Separate physiological roles of specific and non-specific DNA binding of HU protein in *Escherichia coli*

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SUMMARY

Conserved in bacteria, the histone-like protein HU is crucial for genome organization and expression of many genes. It binds DNA regardless of the sequence and exhibits two binding affinities in vitro, low-affinity to any B-DNA (non-specific) and high-affinity to DNA with distortions like kinks and cruciforms (structure-specific), but the physiological relevance of the two binding modes needed further investigation. We validated and defined the three conserved lysine residues, K3, K18, and K83, in *Escherichia coli* HU as critical amino acid residues for both non-specific and structure-specific binding and the conserved proline residue P63 additionally for only the structure-specific binding. By mutating these residues in vivo, we showed that two DNA binding modes of HU play separate physiological roles. The DNA structure-specific binding, occurring at specific sites in the *E. coli* genome, promotes higher-order DNA structure formation, regulating the expression of many genes, including those involved in chromosome maintenance and segregation. The non-specific binding participates in numerous associations of HU with the chromosomal DNA, dictating chromosome structure and organization. Our findings underscore the importance of DNA structure in transcription regulation and promiscuous DNA-protein interactions in a dynamic organization of a bacterial genome.

INTRODUCTION

The *Escherichia coli* genome of 1.5-millimeter contour length is condensed and organized into a helical ellipsoid of $\sim 1.0 \mu^3$ volume that together with its associated RNAs and proteins constitute the *E. coli* chromosome (nucleoid) (Fisher et al., 2013; Verma et al., 2019). The concerted action of the intrinsic DNA properties, and many biochemical factors leads to the formation of the chromosome (Verma et al., 2019). Among the biochemical factors, the histone-like protein HU and the structural maintenance of chromosomes (SMC) complex MukBEF have recently emerged as crucial players for the genome organization as a whole (Lioy et al., 2018; Makela and Sherratt, 2020b; Walker et al., 2020). HU also facilitates gene expression (Prieto et al., 2012) and gene regulatory processes (Aki and Adhya, 1997), chromosomal and prophage DNA replication (Hwang and Kornberg, 1992; Montano et al., 2012; Ogura et al., 1990), DNA repair (Kamashev and Rouviere-Yaniv, 2000), as well as site-specific recombination (Zablewska and Kur, 1995). Additionally, HU regulates bacterial motility (Nishida et al., 1997). Thus, HU plays a major role in bacterial physiology. Not surprisingly, HU is ubiquitous in bacterial kingdom and essential in many bacterial species including many pathogens (Bhowmick et al., 2014; Fernandez et al., 1997). Composed of two homologous subunits α and β encoded by *hupA* and *hupB* genes respectively, the *E. coli* HU can exist as three dimeric forms: $HU\alpha_2$, $HU\beta_2$, and $HU\alpha\beta$. The composition of HU varies according to growth cycle, with $HU\alpha_2$ being predominant in the exponential phase and $HU\alpha\beta$ being predominant in the stationary phase while $HU\beta_2$ is nearly undetectable in any phase (Claret and Rouviere-Yaniv, 1997).

$HU\alpha_2$ and $HU\alpha\beta$ bind DNA independent of base pair sequence but exhibits two distinct DNA binding affinities in vitro. They bind with low-affinity to B-form dsDNA but bind with a 100-fold higher affinity to distorted dsDNA with kinks, nicks, gaps, or cruciform structure (Bonney et al., 1994; Kamashev and Rouviere-Yaniv, 2000; Pinson et al., 1999; Pontiggia et al., 1993; Vitoc and Mukerji, 2011). Hereafter, we refer the low-affinity binding as DNA *non-specific* binding and the high-affinity binding to distorted DNA as DNA *structure-specific* binding. HU is a Y-shaped dimer wherein two homo- or heteromeric subunits intertwine to form a α -helical “body” and two extending β -strands or “arms” (Guo and Adhya, 2007; Swinger et al., 2003). In crystal structures of *E. coli* $HU\alpha_2$ or $HU\alpha\beta$ bound to a 20-bp DNA of random nucleotide sequence (Hammel et al., 2016), mostly three surface exposed lysine residues (K3, K18, and K83) effect non-specific binding through electrostatic interactions with the DNA phosphate backbone (phosphate locks). The lysine residues are present on either side of the dimer allowing a single dimer to interact with two DNA segments through each subunit. The non-specific binding

triggers multimerization of HU α_2 dimers, but not of HU $\alpha\beta$ dimers, resulting in three-dimensional intermolecular DNA *bundling* (Hammel et al., 2016). Controlled by ionic strength and pH, the intermolecular hydrogen bonds between residues E34K and K37 dictates the HU α_2 multimerization, potentially functioning as a molecular switch that regulates DNA bundling in an environment dependent manner (Hammel et al., 2016; Remesh et al., 2020).

In crystal structures of HU in a complex with a distorted dsDNA with kinks, a highly conserved proline residue at position 63 (P63) is critical for the structure-specific binding (Guo and Adhya, 2007; Swinger et al., 2003). While the positively charged surface of the two β -arms reach around the opposite faces of the DNA wrapping the minor grooves, the P63 residues at the tip of each β -arm intercalates into bases in the corresponding minor groove. The two kinks, which are 9 bp apart, coincide with the intercalation of P63 residues, suggesting that P63 intercalation induce and/or stabilize the kinks. HU binds using the same structural motif of paired inclined helices to other forms of distorted DNA such as cruciform and nicked DNA (Balandina et al., 2002; Kamashev et al., 1999).

The contributions, if any, of the lysine residues in DNA structure-specific binding, or that of the P63 residue in non-specific binding are so far unresolved. The DNA in the structure-specific complex in the crystal structures was not long enough to allow contacts with the lysine residues on the sides of the HU “body” (Swinger et al., 2003). Also, the electron density of the β arms was not visible in the crystal structures of HU with the 20 bp dsDNA, implying that the P63 residue was not important for the non-specific binding (Hammel et al., 2016), except that P63 residue may stabilize the intermolecular DNA bundling through β -arm- β -arm interactions via the formation of a β -zipper (Remesh et al., 2020). Despite the extensive in vitro characterization, the details of the two DNA binding modes in the HU-dependent chromosome structure and function in vivo remains to be investigated. In this study, we determined specific contribution of different amino acid residues in the structure-specific and the non-specific DNA binding of HU α_2 in variously proposed physiological processes both in vitro and in vivo. We found that processes needing structure-specific binding require both P63 and lysine residues of HU α_2 , but those involving non-specific DNA binding use only the lysine residues corroborating the structural studies. The requirement of both the lysine residues and P63 residue in specific binding provides a more detailed mechanism of structural-specific binding to a distorted DNA in which the β -arm and the P63 residue bend the DNA further because of the lysine mediated phosphate locks further along the DNA on each side. The structure-specific binding, while not necessary

for overall chromosome structure, is critical for the regulation of specific gene expressions by HU. On the contrary, HU α_2 binds to DNA as part of the chromosome structure primarily through non-specific binding as we previously suggested from single cell analysis (Bettridge et al., 2020). Our findings reveal distinct physiological roles of both structure-specific and non-specific DNA binding modes of HU.

RESULTS

Amino acid residues of HU involved in specific and non-specific DNA binding in vitro

The P63 residue in the β -arm of HU is involved in structure-specific binding (Guo and Adhya, 2007; Swinger et al., 2003). To test any additional role of the three conserved lysine residues (K3, K18, and K83) required for non-specific binding in the structure-specific DNA binding, we used purified wild-type HU α_2 and HU α_2 with P63A mutation, K3A, K83 double mutations, or K3A, K18A, K83A triple mutations to follow HU binding to an artificially designed cruciform DNA (Vitoc and Mukerji, 2011) and a 40-bp double-stranded DNA of random sequence. Binding was measured by fluorescence polarization in solution; the reactions were further loaded onto electrophoretic gels to measure the formation of discrete DNA-protein complexes by electrophoretic mobility shift assays (EMSA).

We observed cooperative binding of wild-type HU α_2 to both the linear duplex and cruciform DNA in solution with similar calculated Hill coefficients (h) and dissociation constants (K_d) (non-specific binding: $h = 3.2$ and $K_d = 0.101 \mu\text{M}$; structure-specific binding: $h = 2.8$ and $K_d = 0.096 \mu\text{M}$) (Figure 1). However, the linear duplex did not migrate as discrete retarded species in EMSA, consistent with weak and transitory non-specific binding (Bettridge et al., 2020; Pinson et al., 1999), but the cruciform DNA migrated as four discrete bands, indicative of strong, cooperative binding of each cruciform DNA molecule to four molecules of HU α_2 as suggested previously (Bonney et al., 1994). The P63A mutation reduced the cooperative binding to cruciform DNA with $K_d = 0.149 \mu\text{M}$, $h = 1.6$, validating the importance of P63 in binding to a distorted DNA as suggested by crystal structures of HU-structure-specific DNA complexes (Guo and Adhya, 2007; Swinger et al., 2003), but it did not affect the binding to the linear DNA duplex ($K_d = 0.106 \mu\text{M}$, $h = 3.0$). In contrast, the K3A, K83A double and K3A, K18A, K83A triple mutations not only reduced the cooperative binding to linear duplex, as expected, they also significantly reduced the same to cruciform DNA (Figures 1). These results suggest that beside P63, the lysine residues are also critical for the structure-specific binding of HU, but P63 does not participate in non-specific binding.

Involvement of structure-specific binding of HU in DNA supercoil restraining

HU constrains DNA when supercoiled (Guo and Adhya, 2007). The crystal structures of HU with a distorted (artificially bent) DNA suggest that HU forms negative supercoils by inducing/stabilizing two kinks in DNA. The kinks are 9 bp apart with bend angles of opposite angular orientations, mimicking a negative writhe (Guo and Adhya, 2007; Swinger et al., 2003), suggesting that the negative writhe may be responsible for HU-mediated supercoil retention in DNA. The intercalation of stacked bases by the P63 residues in the two subunits coincides with the site of kinks, indicating that P63 induces and/or stabilizes the kinks. We tested the importance of P63 and the lysine residues for supercoil restraining ability of HU by incubating the wild-type HU α_2 and HU α_2 variant proteins with a relaxed plasmid DNA, made by introducing a nick in one of the strands. After incubation, the nicks were sealed with DNA ligase, the proteins removed, and the resulting DNA topoisomers were resolved on gels by EMSA. We observed the appearance of supercoils at 0.4 μ M with saturation at 1.6 μ M concentrations with wild type HU α_2 (Figure 2). The P63A mutation significantly reduced the appearance of HU dependent supercoils even at the saturating levels of the mutant protein, demonstrating a role of structure-specific binding of HU α_2 in DNA supercoils. We also found the lysine residues played some roles in the process because the K3A-K83A double mutations in HU α_2 also reduced the ability of helping supercoil formation (Figure 2). From the results of DNA binding described above together with the supercoiling assays, we conclude that the DNA structure-specific binding of HU induces negative supercoils in DNA.

Structure-specific binding in specific gene expression: the gal operon

HU binds specifically to the apical region of the DNA segment between two operator sites of the GalR repressor at the *gal* operon in the *E. coli* chromosome (Majumdar and Adhya, 1984). The binding promotes the formation of a DNA loop by DNA-bound GalR-GalR interactions that represses the transcription from the *P2* promoter located within the looped DNA (Lyubchenko et al., 1997). HU binding stabilizes the DNA bending at the apex, occurring due to transient GalR-GalR interactions (Geanacopoulos et al., 2001). The HU binding requires the formation of a cruciform DNA structure generated at the apex (HU binding site or *hbs*) by DNA supercoiling (Figure S1) (Lewis et al., 1999; Lia et al., 2003). The *gal* system has served as a paradigm for the study of DNA structure-specific binding by HU in gene regulatory systems. We tested the importance of P63 and the lysine residues in the *gal* system by examining the ability of purified wild-type and variant HU α_2 proteins to repress *in vitro* transcription from the DNA looping-sensitive *P2* promoter in a supercoiled DNA template (Lewis et al., 1999). We observed that,

compared to the wild-type, the P63A mutation alone dramatically reduced the ability of HU α_2 to repress the transcription (Figures 3A and S2), confirming a critical role of P63 in the cruciform DNA binding in a physiologically relevant system. Similarly, the K3A-K83A and K3A-K18A-K83A mutations also reduced the ability of HU α_2 to repress the transcription (Figures 3A and S2), confirming that lysine residues also contribute to the binding of the cruciform structure.

We also tested the importance of P63 and the lysine residues in the HU-mediated *gal* repression *in vivo*. We replaced the promoter of the *lac* operon in the chromosome with the *gal* promoter segment to create a *gal-lacZ* transcription reporter fusion. We introduced different mutations in the native *hupA* gene that encodes the α subunit and deleted the *hupB* gene that encodes the β subunit. HU α_2 was as capable of repressing the transcription from the *gal* promoter as the HU $\alpha\beta$ heterodimer since the repressed levels of β -galactosidase activity were similar in the strains with wild-type *hup* genes and with $\Delta hupB$ allele producing only HU α_2 (Figure 3B). Deleting both *hupA* and *hupB* genes resulted in an increase in the levels of β -galactosidase activity, consistent with the de-repression of the *P2* promoter due to the absence of the DNA loop (Lewis et al., 1999). The P63A mutation in the *hupA* gene caused nearly 3-fold increase in the levels of β -galactosidase activity, and a P63E substitution completely abolished the repression by HU α_2 (Figure 3B), confirming the *in vitro* results that P63 is a critical amino acid residue in HU α_2 for binding to DNA and to promote the formation of the DNA loop at the *gal* operon. When we mutated the lysine residues individually, the K3A and K83E substitutions showed de-repression similar to that of the P63A, and, in combinations, K3A-K18A, K3A-K83A, K18A-K83A, K3A-K18A-K83A mutations all showed complete or near complete de-repression (Figure 3B) supporting the *in vitro* finding about the contribution of the lysine residues in binding to the *gal* cruciform DNA *in vivo*.

Structure-specific binding also regulate many other genes

After confirming the role of structure-specific binding of HU in the *gal* operon regulation by a DNA loop formation, we investigated the role of structure-specific binding in regulation of other genes in the *E. coli* chromosome. We analyzed transcriptome of the *E. coli* strains harboring wild-type *hup* genes, $\Delta hupA$ $\Delta hupB$ alleles, $\Delta hupB$ allele producing only HU α_2 , or *hupA*P63A $\Delta hupB$ alleles producing the HU α_2 P63A mutant protein. After mapping the RNA reads to the genome, we followed the workflow described by Chen Y. et al (Chen et al., 2016) to identify any differentially expressed genes (DEGs). We used false discovery rate of $\leq 0.5\%$ and $\log_2$ fold change ≥ 1.5 or ≤ -1.5 to define differential gene expression. The $\Delta hupB$ strain producing HU α_2

homodimer showed differential expression of only two genes (Figure 4) compared to the wild-type strain (WT) that most likely produces HU $\alpha\beta$ heterodimers under the experimental growth conditions (Figure S3). These results suggested that HU α_2 was as capable in controlling gene expression as HU $\alpha\beta$. However, we identified 438 DEGs in the $\Delta hupA \Delta hupB$ strain producing no HU when compared to wild-type strain (Figure 4 and Table S2). 203 genes showed higher expression (up-regulation) and 235 genes showed lower expression (down-regulation).

To measure any role of structure-specific binding of HU in DEGs, we compared gene expression between the strains expressing HU α_2 and HU α_2 P63A mutant protein. The strain expressing HU α_2 P63A showed differential expression of 261 genes compared to the strain expressing HU α_2 (Figure 4 and Table S3). 201 DEGs were common in both the HU-deleted strain and in the strain expressing HU α_2 P63A, and they showed differential expression in the same direction, up- or down-regulation, in both strains (Figure 4 and Table S4). These results show that the loss of the HU structure-specific binding is involved in the regulation of these genes. Since the P63A point mutation does not completely disrupt the structure-specific binding (Figure 1), we predicted the remaining genes may not have enough differential gene expression to be detected as DEGs by our criteria, but their differential expression would trend towards the HU-deleted strain. To test the prediction, we constructed the heatmap of normalized read counts (counts per million) for 438 DEGs identified in the HU-deleted strain (Figure 5). We found that the overall expression pattern of these genes in both the HU-deleted strain and in the strain expressing HU α_2 P63A was similar, supporting the prediction that the complete disruption of the binding may account for even more DEGs observed in the HU-deleted host.

Involvement of HU in bacteriophage replication

HU is important for the growth of bacteriophages Mu and P1 – Mu during its vegetative growth in replicative transposition, and P1 during replication in its prophage (plasmid) state (Montano et al., 2012; Ogura et al., 1990). It is known that HU binds to a specific region of Mu DNA at the left end of the prophage genome and promotes the assembly of a higher-order Mu transpososome structure by bending the DNA and/or stabilizing a pre-bent DNA (Montano et al., 2012). The Mu transpososome assembly triggers repeated replicative transposition of Mu DNA needed for phage growth in the *E. coli* host chromosome.

To understand what kind of HU binding occurs in Mu transpososome, we measured plaque-forming efficiency of Mu phage on *E. coli* lawns expressing either HU α_2 or HU α_2 with the mutations (Table 1). The efficiency of plating on the lawns of the *E. coli* strains expressing HU α_2

with K3A or P63A mutation was about one order of magnitude lower than the strain expressing wild-type HU α_2 . But the combination of both mutations rendered the *E. coli* host completely resistant to the Mu growth. These results suggest that HU binding to Mu DNA involves a structure-specific binding involving both P63 and the lysine residues. We also measured the transformation efficiency of the *E. coli* strains expressing wild-type HU α_2 or HU α_2 with mutations using the P1 plasmid DNA, which is a measure of P1 prophage replication. Compared to the wild-type strain expressing HU α_2 , we did not observe any transformants with *E. coli* expressing HU α_2 with the K3A or the P63A mutation (Table 2) suggesting that the structure-specific binding of HU to P1 DNA is an absolute requirement for P1 plasmid replication.

Nature of HU DNA binding in E. coli chromosome structure and function

Like HU involvement in the replicative processes of bacteriophage and plasmid chromosomes, HU has also been reported to influence replication and partitioning of *E. coli* chromosome (Bahloul et al., 2001; Chodavarapu et al., 2008; Dixon and Kornberg, 1984; Jaffe et al., 1997). We investigated whether HU contributes to such processes by DNA binding. First, we examined any impact of mutating P63 and the lysine residues of HU α_2 on its chromosomal association, which is responsible of chromosomal structural maintenance (Lioy et al., 2018; Walker et al., 2020), by following the co-localization of HU α_2 and its variants with the chromosome in vivo in single cells using wide-field fluorescence microscopy. Whereas most of the wild-type HU α_2 and its P63A mutant co-localized very well with the chromosome with a distribution pattern resembling the shape of the chromosome, the HU α_2 with the triple lysine mutations K3A-K18A-K83A did not co-localize with DNA in the chromosome (Figures 6A and 6B). These results, together with in vitro DNA binding assays described above (Figure 1), suggest that non-specific DNA binding, which requires only the conserved lysine residues but not P63, is the primary mode of overall HU-DNA interactions in the chromosome. These results do not rule out any role of structure-specific bindings at some limited sites in the chromosome.

Second, we also investigated any influence of HU-binding on chromosome numbers per cell and chromosome morphology (width and length) along with associated cell length and cell morphology using fluorescence imaging, and if so, what kind of DNA binding is involved. Although we observed that the average cell length of the strain with HU α_2 P63A was not much different from that of the strain with wild-type HU α_2 , the distribution of the cell length varied significantly among individual cells (Figure 6C). The coefficient of variation in the mutant was about 66% compared to 38% in the wild-type. About 33% of the cells expressing HU α_2 P63A

were longer than 5 μm , 17% of which were longer than 10 μm , compared to 24% of cells expressing wild-type HU α_2 being longer than 5 μm with 6% of those being 10 μm longer.

Third, a more drastic effect of the P63A mutation occurred on the number of chromosomes per cell; about 26% of the mutant cells were anucleate (Figure 6C). The average length of the anucleate cells was 2.6 μm , about two times smaller than that of nucleated cells. Wild-type cells produced only 2% anucleate cells. We note that the anucleate cells are in small part due to the mVenus tag because the strain without tag produced no anucleate cells (Figure 6C). Nonetheless, the P63A mutation increased the fraction of anucleate cells by ten-fold compared to the wild-type HU α_2 with the tag. The results suggest a role of HU structure-specific binding in chromosome partitioning. The triple lysine mutations K3A-K18A-K83A in HU α_2 resulted in even higher fraction of anucleate cells (45%), presumably, adding to further loss of DNA-structure-specific binding.

After merging the images of individual cells containing at least two, or more, segregated chromosomes into a single aggregate image, the merged chromosome images showed separation between chromosomes in the strain expressing wild-type HU α_2 (Figure 6B), suggesting that the chromosomes localize in the same position in each cell. The merged chromosomes, however, had no gap in between for the strain expressing HU α_2 P63A or HU α_2 with K3A-K18A-K83A mutations (Figure 6B), indicating that the disruption of HU-DNA interactions cause mis-localization of the chromosomes. Taken together, the production of anucleate cells and mis-localization of the chromosome in case of the P63A mutation alone suggest that the DNA structure-specific binding of HU α_2 is critical for proper chromosome positioning and partitioning in *E. coli*. We did not observe a significant difference in average chromosomal area or average chromosomal length between the strains expressing wild-type HU α_2 and mutant HU α_2 P63A (Figure 6C). The average chromosome width in the strain expressing HU α_2 P63A, however, was significantly less than that of the strain with wild-type HU α_2 (~ 0.4 μm compared to ~ 0.5 μm , p -value < 0.05 , Figure 6C). These results show that the loss of structure-specific HU-DNA interactions are not only important for proper chromosome positioning and partitioning but also for maintaining the chromosome morphology.

DISCUSSION

In *E. coli*, HU displays two kinds of DNA binding: (a) We have previously shown that the HU protein forms a crystal complex with a 20-bp DNA duplex of random sequence in which three

highly conserved, surface-exposed lysine residues K3, K18, and K83 in the protein form ionic bonds with DNA phosphate (Hammel et al., 2016). In this report, we biochemically confirm the non-specific DNA binding of HU α_2 . (b) The physiologically relevant structure-specific DNA binding of HU was previously shown to require a proline residue P63 in the β -arm by crystal structure analysis (Guo and Adhya, 2007; Swinger et al., 2003). We also biochemically demonstrate that the structure-specific binding not only require the P63 but also involve the surface exposed lysine residues. We postulate that in the structurally specific binding of HU α_2 to a distorted DNA, while the P63 residues in the tip of the β -arms recognize the bent DNA helices, the non-specific contacts between the encompassing DNA and lysine residues on both sides of the bound HU dimer provides stability and sharper DNA bending (Figure 7).

HU has a multifarious physiological role in *E. coli* involving DNA (Verma et al., 2019). Here we have assigned which type of HU binding to DNA – non-specific or structure-specific – is involved in many of its known physiological roles. Since HU α_2 homodimer fulfils most of the roles of HU $\alpha\beta$ heterodimer, we approached the objective by using HU α_2 and its variants for technical ease by mutating the P63 and/or lysine residues in the *hupA* gene while deleting the *hupB* gene in the chromosome.

- (i) We have shown here that in restraining DNA supercoils, HU uses structure-specific binding, presumably by binding at any constituent cruciform structures.
- (ii) The deletion of HU has previously been shown to cause differential expression of many genes in *E. coli* (Prieto et al., 2012), but it is unclear how HU brings about the changes in gene expression - whether through HU-dependent chromosomal structural changes mediated by non-specific binding or by the formation of local higher-order DNA structures by structure-specific HU binding. We found that the DNA structure-specific binding, while not necessary for the overall chromosome structure, is critical for the regulation of gene expression by HU. We hypothesize that the structure-specific binding occurring at limited sites in the genome controls gene expression at transcription level by allowing a local DNA loop formation in the promoter region - repressosome or enhanceosome formation for gene repression or gene activation respectively (Geanacopoulos et al., 2001; Panne, 2008). For the assembly of repressosomes or enhanceosomes at specific genes, HU may recognize and bind with higher affinity to cruciform structures arising from inverted repeat sequences (IRS) in the negatively

supercoiled *E. coli* genome (Sinden et al., 1980). The HU binding site (*hbs*) in the *gal* operon is an IRS, and negative supercoiling is required for repression of the *gal* operon (Lewis et al., 1999). We have confirmed here that the structure-specific HU binding is required for DNA loop formation leading to the *gal* operon repression. Since the P63 residue is also critical for the supercoil restraining ability of HU, the binding of HU to cruciform structures near genes may alternatively restrain negative supercoiling. There is indirect evidence suggesting that HU restrains transcription-induced supercoiling in the chromosome (Berger et al., 2016). The loss of HU binding can free these supercoils that, in turn, can alter transcription. Therefore, it is possible that drastic effect of the P63A mutation we observed on gene expression may have resulted from the formation of free supercoils. But the differentially expressed genes were not enriched for supercoiling sensitive genes identified previously (Peter et al., 2004) (Figure S4), contradicting the idea of supercoiling-mediated regulation of gene expression by HU.

- (iii) Although the structure-specific DNA binding of HU does not participate in the genome organization, as reported above, and discussed below, we have shown that HU-deleted strains are defective in proper chromosome partitioning giving rise to anucleate cells. The HU effect on chromosome partitioning is due to structure-specific binding as HU α_2 with the P63 mutation showed chromosome partitioning defect. When studying the effect of HU in gene regulation, we observed that the genes encoding integration host factor (IHF) and DNA-binding protein from starved cells (Dps), plus the DNA gyrase inhibitor protein SbmC were down-regulated, whereas genes encoding MukB and the ParC subunit of DNA Topoisomerase IV were up-regulated upon the deletion of HU genes or by the disruption of the structure-specific binding in HU α_2 . MukB is the DNA binding subunit of the SMC complex MukBEF, which upon over-expression has recently been shown to form an axial core, resulting in the lengthwise compaction and organization of the *E. coli* chromosome (Makela and Sherratt, 2020a). TopoIV is a decatenase required for the segregation of the sister chromosomes. Additionally, the MukBEF complex and TopoIV physically interact, and the interaction likely coordinates timely segregation of the newly replicated chromosomes (Nolivos et al., 2016). Thus, we believe that the formation of anucleate cells and chromosome mis-positioning we observed due to the disruption of the structure-specific binding of HU may have resulted from the over expression of MukB and TopoIV. Here the HU structure-specific

binding indirectly impacts the chromosome partitioning through alteration of gene expression.

(iv) We found that only the non-specific binding participated in a major role of HU - its association with the chromosomal DNA that dictates the chromosome structure and organization. By tracking single molecule analysis in live cells, we have previously demonstrated (Bettridge et al., 2020) and further confirmed here that the non-specific binding of HU is responsible for this interaction with rapid association and dissociation kinetics that keep most HU within the nucleoid. Mutating the three lysine residues to alanine dramatically altered the interacting dynamics of HU α_2 but mutating P63 to alanine did not have any effect (Bettridge et al., 2020). We do not rule out that structure-specific binding of HU at some defined sites may also play some architectural role in the chromosome as well.

In summary, our findings demonstrate that non-specific and structure-specific DNA binding modes of HU plays distinct roles: (a) HU has many targeted roles in various DNA metabolic processes like gene transcription, DNA supercoiling, chromosome positioning and partitioning. We have shown that some of these processes are direct, and some are indirect effect of structure-specific binding of HU to DNA. (b) The non-specific but highly dynamic binding of HU to chromosomal DNA organizes the chromosome that include wide-spread DNA-DNA contacts. The non-specific binding also keeps HU mostly as a component of the chromosome. HU is known to promote DNA-DNA contacts in the megabase pair range (Lioy et al., 2018; Walker et al., 2020) but the mechanism is unknown. We have previously shown that the non-specific DNA binding triggers multimerization of HU α_2 (Hammel et al., 2016; Remesh et al., 2020). There is one HU dimer every ~150 bp of the chromosomal DNA based on the estimated abundance of 30,000 HU dimers per cell during the exponential growth phase (Ali Azam et al., 1999), and most of the HU dimers are in the form of HU α_2 (Claret and Rouviere-Yaniv, 1997). By consolidating these multiple lines of evidence, we argue that the multimerization of HU α_2 molecules non-specifically bound to numerous positions on the chromosomal DNA brings distant genomic loci together. The rapid interacting dynamics of HU with DNA (Bettridge et al., 2020) may be necessary for the newly replicated genome to dynamically reorganize as the genome progressively segregate during DNA replication (Nielsen et al., 2006). Most target specific DNA binding proteins use base sequences specific motifs; their non-specific DNA binding abilities are relegated to as background noise. In contrast, the non-specific DNA binding

activity of HU plays a critical role in chromosome architecture and organization, as also confirmed by recent our studies probing the 3D genome of *E. coli* using independent approaches (in preparation), making HU a unique DNA binding protein.

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TABLES

Table 1. Effect of various mutations in HU on the Mu phage growth.

Strain	Genotype	² PFU/ml
MG1655	WT	6.6×10^8
SCV129	HU α_2	6.3×10^8
SCV130	HU α_2 - <i>mVenus</i> ¹	5.3×10^8
SCV142	K3A	5.7×10^7

SCV140	K18A	3.2×10^8
SCV108	K83A	3.2×10^8
SCV106	P63A	2.1×10^7
SCV111	K3A K83A	0
SCV133	K83A P63A	0
SCV144	K3A K18A K83A	0

¹ Mutations indicated are in HU α_2 -*mVenus*.

² Plaque forming units.

Table 2. Effect of various mutations in HU on the P1 phage (plasmid state) growth.

Strain	Genotype	CFU ² /μg pUC18	CFU/μg pSP102
MG1655	WT	2.9×10^9	9.5×10^9
SCV18	HU β_2	2.6×10^9	3.0×10^{10}
SCV129	HU α_2	4.5×10^9	1.8×10^{10}
SCV27	HU null	3.6×10^9	0
SCV130	HU α_2 - <i>mVenus</i> ¹	4.4×10^9	3.9×10^{10}
SCV144	K3A K18A K83A	4.7×10^9	0
SCV106	P63A	8.9×10^9	0
SCV111	K3A K83A	5.1×10^9	0

¹ Mutations indicated are in HU α_2 -*mVenus*¹.

² colony forming units

FIGURE LEGENDS

Figure 1. Amino acid residues of HU involved in specific and non-specific DNA binding in vitro

Binding of purified wild-type HU α_2 or its variants to a 6-carboxy fluorescein labeled 40-bp double stranded DNA of random sequence (B-DNA) or 40-bp artificially designed cruciform DNA. On the left, each data point on the Y-axis represents the milipolarization (mP) units measured by fluorescence polarization. The line represents the best fit of the data points using the hill slope equation. On the right, the binding reactions of wild-type HU α_2 analyzed by electrophoretic

mobility shift assay. Lanes 1 to 10 contain 0.01, 0.03, 0.06, 0.12, 0.25, 0.5, 0.1, 0.2, 0.4, and 0.8 μ M wild-type HU α_2 . Lane 11 contains only the DNA.

Figure 2. Involvement of structure-specific binding of HU in DNA supercoil restraining

The ability of wild-type HU α_2 protein or its variants to induce negative supercoils in singly nicked plasmid DNA. A 11090-bp singly nicked plasmid was incubated with the proteins at indicated concentrations and then resealed using T4 DNA ligase. After removing the protein, the DNA was resolved on the 0.8% agarose gel for 18 hours and stained with SYBER gold.

Figure 3. The role of structure-specific DNA binding of HU in the transcription of galactose utilization genes

A. The in vitro transcription from the DNA looping sensitive *galP2* promoter in the presence of 200 nM GalR and wild-type HU α_2 protein or its variants. Each data point represents the intensity of the *galP2* transcript band in the gel in Figure S2 after normalizing by the RNA 1 transcript band intensity in the same lane.

B. β -galactosidase activity of the *gal-lacZ* transcription reporter in *E. coli* strains harboring wild-type *hup* genes (WT), $\Delta hupA \Delta hupB$ alleles (HU null), $\Delta hupA$ allele producing only HU β_2 , and $\Delta hupB$ allele producing only HU α_2 , HU α_2 -mVenus, or HU α_2 -mVenus with indicated mutations.

Figure 4. Involvement of structure-specific binding of HU in global gene expression

Volcano plots showing differential gene expression in the *E. coli* strain harboring $\Delta hupA \Delta hupB$ alleles (HU null) compared to the strain harboring wild-type *hup* genes (WT), in the strain harboring $\Delta hupB$ allele producing only HU α_2 (wt HU α_2) compared to WT, and in the strain harboring $\Delta hupB hupA P63A$ -mVenus allele producing HU α_2 P63A-mVenus (HU α_2 P63A) compared to the strain harboring $\Delta hupB$ allele producing only HU α_2 . y-axis represents inverse of Log10 value of false discovery rate (FDR), and x-axis represents Log2 value of the fold-change (Log2FC) in the transcript abundance. Horizontal dotted line represents 0.05 FDR cut off and vertical dotted line represents 1.5 log2FC cut off. DEGs denotes differentially expressed genes. Venn diagram showing overlap in DEGs between HU null versus WT and HU α_2 P63A vs wt HU α_2 conditions.

Figure 5. Overall expression pattern of genes identified as differentially expressed genes in the HU null strain

Heat map of normalized reads counts (counts per million) of 438 genes identified as

differentially expressed genes in the HU null strain. Each column represents a single replicate of the strain indicated. Each row represents a single gene.

Figure 6. Nature of HU DNA binding in *E. coli* chromosome structure and function

A. In vivo chromosome association of wild-type HU α_2 -mVenus or its variants. The fluorescence signal of mVenus is falsely colored as green. The DNA was stained with Hoechst 33342, and the fluorescence signal is falsely colored as red. The fluorescence images were obtained after subtracting the corresponding binary mask images from the original images.

B. The aggregate images of the chromosome and the HU α_2 -mVenus or its variants, obtained by merging the fluorescence images of at least five individual cells with two or more well-segregated chromosomes.

(C) Violin plots showing the distribution of cell length, the number of chromosomes per cell, chromosome area, width, and length of cells expressing HU α_2 , HU α_2 -mVenus, or HU α_2 -mVenus variants. The bar graph shows the percentages of anucleate cells.

Figure 7. Cartoon representation of the non-specific and structure-specific DNA binding modes of HU.

Two homo- or heteromeric subunits of HU are depicted as blue and purple. Critical amino acids for DNA binding are depicted as red circles. DNA is depicted in gold. In the non-specific binding mode to any B-DNA, three highly conserved lysine residues K3, K18, and K83 form electrostatic interactions with the phosphate backbone as shown previously by crystal structures and confirmed biochemically in this study. The bound DNA is mostly in the straight conformation. A single HU dimer can bind to two separate DNA molecules or two different segments of the same long DNA molecule. The beta arms do not contribute to the non-specific binding and therefore represented here in the faded color.

In the structure-specific binding mode to DNA with distortions, the beta arms recognize a common structural motif of bent DNA helices wherein the proline residue P63 at the tip of the arms intercalates between bases at the site of kinks. As biochemically demonstrated in this study, the lysine residues contribute significantly to the binding. We propose that the non-specific interactions between the lysine residues and the phosphate backbone further along the DNA on both sides stabilize the binding and bends the DNA more sharply.

METHODS

Key resource table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial strains		
<i>Escherichia coli</i> K12 MG1655	CGSC	CGSC#: 6300
SCV18 ($\Delta hupA$)	(Remesh et al., 2020)	
SCV27 ($\Delta hupA \Delta hupB$)	(Remesh et al., 2020)	
SCV36 (MG1655) <i>hupA-mVenus</i>	This study	
SCV123 (MG1655) <i>hupA-mVenus</i>	This study	
SCV129 (MG1655) <i>hupB11</i> (Cm ^R)	This study	
SCV130 (123) <i>hupB11</i> (Cm ^R)	This study	
SCV106 (SCV36) <i>hupA P63A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV107 (SCV36) <i>hupA P63E-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV108 (SCV36) <i>hupA K83A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV109 (SCV36) <i>hupA K83E-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV111 (SCV36) <i>hupA K3A K83A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV131 (SCV36) <i>hupA K18A K83A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV133 (SCV136) <i>hupA K83A P63A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV140 (SCV36) <i>hupA K18A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV141 (SCV36) <i>hupA K3A K18A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV142 (SCV36) <i>hupA K3A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV144 (SCV36) <i>hupA K3A K18A K83A-mVenus</i> <i>hupB11</i> (Cm ^R)	This study	
SCV157 (MG1655) <i>bla-gal-lacZ</i>	This study	

SCV160 (SCV130) <i>bla-gal-lacZ</i>	This study
SCV163 (SCV27) <i>bla-gal-lacZ</i>	This study
SCV161 (SCV18) <i>bla-gal-lacZ</i>	This study
SCV159 (SCV129) <i>bla-gal-lacZ</i>	This study
SCV169 (SCV142) <i>bla-gal-lacZ</i>	This study
SCV168 (SCV140) <i>bla-gal-lacZ</i>	This study
SCV165 (SCV108) <i>bla-gal-lacZ</i>	This study
SCV225 (SCV109) <i>bla-gal-lacZ</i>	This study
SCV164 (SCV106) <i>bla-gal-lacZ</i>	This study
SCV224 (SCV107) <i>bla-gal-lacZ</i>	This study
SCV229 (SCV141) <i>bla-gal-lacZ</i>	This study
SCV166 (SCV111) <i>bla-gal-lacZ</i>	This study
SCV227 (SCV131) <i>bla-gal-lacZ</i>	This study
SCV167 (SCV133) <i>bla-gal-lacZ</i>	This study
SCV170 (SCV144) <i>bla-gal-lacZ</i>	This study

Plasmids

pUC18	(Norrandar et al., 1983)
pSP102	(Pal et al., 1986)
pCG09	(Hayama et al., 2013)

Oligonucleotides

PCR primers	Integrated DNA Technology	See Table S1
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Chemicals, Peptides, and Recombinant Proteins

Hoechst 33342	Invitrogen	Cat# H3570
TRIzol reagent	Invitrogen	Cat# 15596026
TURBO DNA-free Kit	Invitrogen	Cat# AM1907
Wild-type HU α_2	Custom synthesis by GenScript	
HU α_2 with P63A mutation	Custom synthesis by GenScript	
HU α_2 with K3A K83A double mutation	Custom synthesis by GenScript	
HU α_2 with K3A K18A K83A double mutation	Custom synthesis by GenScript	

Software and Algorithms

MATLAB	MathWorks	https://www.mathworks.com/products/matlab.html
ImageJ	(Schneider et al., 2012)	https://imagej.nih.gov/ij/

Bacterial strains and growth conditions

Bacterial strains and primers are listed in Key Resources Table and Table S1, respectively. All strains were derivatives of *E. coli* K12 MG1655 (Blattner et al., 1997), and were constructed by Lambda red recombineering (Yu et al., 2000) using the plasmid pSIM6 (Datta et al., 2006). The SCV36 strain harboring *hupA-mVenus* fusion served as the parent strain for the strains harboring point mutations in the *hupA* gene. The *hupA-mVenus* fusion was constructed in two steps. In the first step, a pBAD-ccdB-kan genetic element that expresses CcdB toxin under the arabinose inducible promoter pBAD and encodes a kanamycin resistance gene was inserted after the stop codon of the *hupA* open reading frame. The recombinants were selected on LB agar plates containing 1% glucose and 30 $\mu\text{g ml}^{-1}$ kanamycin. The recombinants were verified by PCR using the primers that bind outside the *hupA* gene as well as by the inability of the recombinants to grow on LB agar plates supplemented with 0.02% arabinose that induces the expression of the toxin CcdB. In the second step, a synthetic double-stranded DNA (synthesized by Integrated DNA Technologies) encoding the *hupA* gene, the glycine-serine-isoleucine (GSI) peptide linker, and yellow fluorescent protein mVenus was recombined. The recombinants were selected on LB agar with 0.02% arabinose and subsequently were verified by Sanger sequencing. After sequence verification, the plasmid pSIM6 was removed by repeatedly growing the strain at 37 $^{\circ}\text{C}$ and selecting for ampicillin sensitivity. The point mutations were introduced using same steps; first inserting the pBAD-ccdB-kan element and then recombining the synthetic dsDNA encoding the *hupA-mVenus* fusion gene with desired mutations in the *hupA* gene. The *hupB11* allele (Wada et al., 1988) that has the *hupB* region between EcoRV and AatI sites replaced with a 1.4 kb HaeII DNA fragment containing the chloramphenicol-resistance (Cm^{R}) gene of the pACYC184 plasmid was transferred from a strain of our lab collection using P1 phage transduction.

Cells were grown in M63 minimal media supplemented with 0.1% glycerol (v/v), 10 $\mu\text{g ml}^{-1}$ thiamine, and 0.5 mg ml^{-1} casamino acids. For RNA extraction and fluorescence microscopy, cells were grown overnight, diluted 1000-fold and grown to A_{600} of 0.4-0.6.

Protein purification

Wild-type HU α_2 protein and HU α_2 protein with P63A mutation, K3A, K83 double mutations, or K3A, K18A, KA83 triple mutations were purified by GenScript Biotech Corporation. 6x-histidine tag containing Enterokinase cleavage site was placed at the N-terminus. Proteins were expressed in *E. coli* strain BL21(DE3) and purified with >95% purity using Ni-affinity column followed by Q-Sepharose and size-exclusion chromatography. The proteins were stored in the buffer containing 50 mM Tris HCl pH 8.0, 150 mM NaCl, 10% Glycerol. The tag was removed by histidine tagged Bovine Enterokinase (GenScript Biotech Corporation) before using the proteins for DNA binding assays.

DNA binding assays

The 6-carboxy fluorescein (6-FAM) labelled duplex DNA synthesized by Integrated DNA Technologies were used for DNA binding. The following oligonucleotides were used for synthesizing the duplex DNA.

B-form DNA of random sequence:

CCGACTAAGTACATGTGAGAATTTTGCTGCCTTCGAACCT and

/56-FAM/AGGTTCTGAAGGCAGCAAAATTCTCACATGTACTTAGTCGG

Cruciform DNA:

CCTAGCAAGGGGCTGCTACCTTTGGTAGCAGCCTGAGCGGTGG and

/56-FAM/CCACCGCTCAACTCAACTGCTTTGCAGTTGAGTCCTTGCTAGG

The binding assays were carried out in the 100 μ l reaction volume containing 50 mM Tris-HCl pH 8.0, 30 mM NaCl, 1 nM DNA, and varying concentrations of the protein. The reactions were incubated in 6 mm x 50 mm glass test tubes at room temperature for 10 min and then fluorescence polarization measurements were taken in the BeaconTM 2000 instrument at 25°C.

Subsequently, 20 μ l reaction volume was loaded onto 6% DNA retardation gels (Invitrogen), and electrophoresis was carried out at room temperature in 0.5x Tris borate buffer, pH 8.0. The gels were imaged using Bio-Rad ChemiDoc MP Imaging System. The milipolarization (mP) units were plotted in GraphPad Prism v9. The data points were fitted using the Hill slope equation $Y = B_{max} \times X^h \div (K_d^h + X^h)$ wherein B_{max} is the maximum binding in the mP units, X is the protein concentration, h is the hill slope, and K_d is the protein concentration needed to achieve the half-maximum binding at equilibrium.

Transcription Assays

In vitro transcription reactions were carried out as described earlier (Lewis, 2003). Supercoiled

plasmid pSA850 (40 nM) was preincubated at 37°C for 5 min with 20 nM RNA polymerase and 200 nM GalR and/or varying concentrations of wild-type HU α_2 or its mutant variants in a total reaction volume of 50 μ l containing transcription buffer (20 mM Tris acetate/10 mM Mg acetate/50 mM NaCl) supplemented with 1 mM DTT, 1 mM ATP, and 0.8 units recombinant ribonuclease inhibitor (rRNasin. As needed, were added before RNA polymerase. Transcription reactions were initiated by adding nucleotides to a final concentration of 0.1 mM GTP and CTP, 0.01 mM UTP, and 5 μ Ci [α -32P] UTP (1 Ci = 37 GBq). The reactions were incubated for an additional 10 min before they were terminated by the addition of an equal volume (50 μ l) of loading dye (90% formamide/10 mM EDTA/0.1% xylene cyanol/0.1% bromophenol blue). Samples were heated to 90°C for 2–3 min, chilled, then loaded on an 8% sequencing gel and electrophoresed at a constant power of 60 W in TBE (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3). The RNAI transcripts (106 and 108 nts) were used as an internal control to normalize the relative amount of transcript from *P2* promoter.

Supercoiling assay

The singly nicked pCG09 plasmid was prepared by incubating CsCl purified negatively supercoiled pCG09 (60 μ g) in 600 μ l of 1X NEB Smart Cut buffer with the nicking endonuclease Nb.BbvCI (NEB, 8.5 units) at 37 °C for 30 min. The plasmid was purified by phenol: chloroform: isoamyl alcohol extraction, recovered by standard ethanol precipitation, and dissolved in 1X buffer containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, and 0.05 % (v/v) IGEPAL 630 (Sigma). Wild-type HU α_2 protein and its variants were incubated with singly nicked pCG09 plasmid for 5 min in a buffer containing 50 mM HEPES-KOH (pH 7.5), 20 mM KCl, 30 mM NaCl (from protein stocks containing 150 mM NaCl), 10 mM DTT, 5 mM MgOAc, 7.5 % glycerol, 2 mM ATP-Mg and 5 ng/ μ L tRNA. T4 DNA ligase was added to seal the nick. The reactions were stopped by addition of NaCl and EDTA followed by deproteinization with SDS and proteinase K for 30 min at 37°C. Topoisomers were resolved on a 0.8 % agarose gel at room temperature and 23 volt for 18 hours. Gels stained with SYBR Gold and imaged by Typhoon scanner.

β -galactosidase assay

The *gal-lacZ* reporter was constructed by replacing the chromosomal region of MG1655 starting from the *lacI* up to the start codon of the *lacZ* with the DNA fragment of the plasmid pSA813 containing ampicillin resistance gene *bla*, the *gal* promoter, and the *galE* gene. The *gal-lacZ* reporter was transferred to other strains using P1 phage transduction.

β -galactosidase assay was carried out using standard protocol. Briefly, cells were diluted in Z buffer and permeabilized by adding 100 μ l chloroform and 50 μ l 0.1% sodium dodecyl sulfate. The reaction was initiated by adding 0.2 ml of 4 mg/ml o-nitrophenyl- β -D-galactoside in 0.1M phosphate buffer (pH 7.0), incubated at 28°C, and stopped after sufficient yellow color developed by adding 0.5 ml 1M Na₂CO₃. 1 ml of the reaction was transferred to an eppendorf tube, spun at maximum speed to remove debris and chloroform, and the absorbance at 420 nm was recorded for each tube. The following equation was used to calculate units of enzyme activity: $1000 \times (A_{420}) / (A_{600} \times T \times V)$ where T is the time of reaction in minutes and V is the volume of cells used in the reaction in milliliters.

RNA sequencing

For total RNA isolation, a frozen pellet of cells was resuspended in 1 ml TRIzol reagent (Life Technologies) to homogenization and incubated at room temperature for 5 min. To the resuspension, 0.2 ml chloroform was added and mixed by inverting the tube for 15 seconds. The mixture was incubated at room temperature for 10 min and then centrifuged at 20,000 $\times$ g for 10 min at 4°C. After centrifugation, ~0.6 ml of the upper phase was transferred to a new centrifuge tube containing 0.5 ml isopropanol. The mixture was incubated at room temperature for 10 min and then centrifuged at 20,000 $\times$ g for 15 min at 4°C. After centrifugation, the supernatant was discarded and the pellet was washed twice with 1 ml 75% ethanol, made with Diethyl pyrocarbonate (DEPC)-treated water, by centrifugation at 13,544 $\times$ g, for 5 min at 4°C. After the second wash, the tube was left open for 10–15 min at room temperature to dry the pellet. To the pellet, 50 μ l DEPC treated water was added and the tube was left at 37°C for 10–15 min and then the pellet was fully resuspended using a pipette. DNA was removed using TURBO DNA-free Kit (Invitrogen). Quality of total RNA was determined by electrophoresis on the TapeStation system (Agilent). Paired-end sequencing libraries were prepared with 2.5 μ g of total RNA using Illumina TruSeq Stranded Total RNA library prep workflow with Ribo-Zero. Samples were pooled and sequenced on HiSeq4000 with read length of 150. Samples were barcode demultiplexed allowing one mismatch using Bcl2fastq v2.17. The reads were trimmed for adapters and low-quality bases using Cutadapt software. Alignment of the reads to the annotated transcriptome of *E. coli* K12 MG1655 was done using STAR. Transcript abundances were calculated by RSEM, and differential expression analysis was done using the glmTreat function of edgeR. We identified differential expression based on false discovery rate (FDR) cut off of 0.05 and log₂ (log with base 2) fold change of 1.5. The RNA-Seq data have been deposited to the Gene Expression Omnibus (GEO) data base

and can be accessed with the GEO accession number GSE175465.

Mu phage growth assay

Strains were first grown overnight in LB broth. The overnight cultures were 100 times diluted into LB broth supplemented with 1mM CaCl_2 and 2.5 mM MgCl_2 and grown to A_{600} between 0.3-0.4. Cells were centrifuged and resuspended such that A_{600} is 1.0. 100 μl cells were mixed with 10 μl serially diluted phage lysate and incubated at 37°C for 15 min. The mixture was added to 3 ml of 0.7% top agar and poured onto Terrific Broth agar plates. The next day, plaque were counted by eye. The plaque forming units/ml was calculated as average number of plaques divided by dilution of the phage lysate times volume of the phage lysate.

P1 plasmid transformation assay

Electrocompetent cells prepared using standard protocol were transformed with pUC18 or pSP102 plasmid DNA by electroporation. The transformants were selected on LB agar plates containing appropriate antibiotic. The transformation efficiency was calculated as colony forming units per microgram plasmid DNA.

Fluorescence microscopy

1 ml bacteria culture was stained with 10 $\mu\text{g ml}^{-1}$ Hoechst 33342 for 10 min and centrifuged at 1000 x g for 3 min at room temperature. All supernatant was removed except the volume in microliters equal to $A_{600} \times 200$. The resuspended cells were stained with 15 $\mu\text{g ml}^{-1}$ FM464 for 10 min. Cells were then spotted onto 35 mm Poly-D-Lysine coated glass petri dish (MatTek Life Sciences) and covered by M63 glycerol 1% (w/v) agarose pad. Z- sections were collected using DeltaVision imaging system (GE Healthcare) equipped with CoolSNAP_HQ2 camera. The pixel size of each image was 0.064 $\mu\text{m} \times 0.064 \mu\text{m} \times 0.2 \mu\text{m}$. mVenus fluorescence was detected using Fluorescein isothiocyanate (FITC) filter (excitation: 475/28; emission: 525/48) with 100% light transmission. Hoechst 33342 was detected using 4',6-diamidino-2-phenylindole (DAPI) filter (excitation: 390/18; emission: 435/48) with 50% light transmission. FM464 was detected using Tetramethylrhodamine filter (excitation: 542/27; emission: 597/45) with 100% light transmission. Exposure time for Hoechst 33342 and FM464 was 0.5 s, and for mVenus was 0.2 s. The images were deconvoluted using the recommended method in the SoftWoRx software. Image quantitation was performed in ImageJ using the middle section of the z-stack. Cell length was manually measured using FM464 fluorescence. Chromosome length and chromosome width were measured as major axis and minor axis, respectively.

To generate the aggregate images of the chromosome and the HU α_2 -mVenus, the images of individual cells were cropped and rotated such that the long axis of cells corresponded to the x-axis and the middle of the cell corresponded to the axis origin. Then, the chromosome and HU α_2 -mVenus channels were split, channel images of individual cells were concatenated, and aggregated into a single image using 2-D sum intensity projection. The aggregate images were cropped to 12.99 micron long and 4.67 micron wide. For better visual comparison, the pixel intensity of the aggregate images was normalized in MATLAB by the maximum pixel intensity of that image, and then multiplied by 255. To account for intensity and noise differences, the aggregate images were further normalized in MATLAB using the aggregate image of WT chromosome as template and using histogram matching combined with gradient information (Sintorn et al., 2010).

Figure 1

B-DNA

Cruciform DNA

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

A

B

C

Figure 7

A.

Non-specific

B.

Structure-specific

Figure S1

Figure S1. The presence of a potential cruciform structure within the HU binding site (*hbs*) at the *gal* operon.

The inverted repeat sequence is highlighted in red and green. Black dots represent nucleotides in physical contact with HU in a supercoiled DNA template as determined previously by chemically converting HU into a nuclease (Aki and Adhya, 1997). +1 is the transcription start site.

Figure S2

Figure S2. Effect of point mutations in HU on the in vitro transcription from the *gal* promoters. Lanes 1 and 13: no GalR or HU; Lanes 2 and 14: 200 nM GalR, Lanes 3 to 7: 200 GalR and 50, 100, 200, 400, and 800 nM wild-type HUα₂; Lanes 8 to 12: 200 GalR and 50, 100, 200, 400, and 800 nM HUα₂ P63A; Lanes 15 to 19: 200 GalR and 50, 100, 200, 400, and 800 nM HUα₂ K3A K83A; Lanes 20 to 24: 200 GalR and 50, 100, 200, 400, and 800 nM HUα₂ K3A K18A K83A.

Figure S3

Figure S3. Levels of α and β subunits of HU during cell growth

Strains expressing HU α -mVenus or HU β -mVenus were first grown in standard test tubes in M63 minimal media supplemented with 0.1% glycerol (v/v), 10 $\mu\text{g ml}^{-1}$ thiamine, and 0.5 mg ml^{-1} casamino acids. Cultures were diluted 100-fold into fresh media, and 1-ml added to 24-well plates with glass bottom (Greiner Bio-One). Cell growth and fluorescence were measured in Tecan Spark plate reader in real time every 30 min. Please note that optical density at 600 nm measured by the plate reader is not the same as that measured by standard spectrophotometer.

Since HU α and HU β have a higher affinity with each other than between themselves (Ramstein et al., 2003), WT strain likely produces HU predominantly in the form of the HU $\alpha\beta$ heterodimer throughout the growth cycle.

Figure S4

Figure S4. Supercoiling sensitive genes.

Pie chart showing supercoiling sensitive genes ((Peter et al., 2004) as percentage of total *E. coli* K12 MG1655 genes or differentially expressed genes identified in the absence of HU.

Table S2. Differentially expressed genes in the HU null strain relative to strain expressing wild type *hup* genes.

Enterz			
Gene ID	Symbol	logFC	FDR

944844	argF	5.56050871	0.01317872
949025	carA	5.29535781	0.00290574
948774	argI	4.85587594	0.0246394
945370	bioB	4.59070294	0.00263982
945384	bioF	4.54460138	0.00306724
948455	argC	4.42217088	0.02487785
948981	artJ	4.41514064	0.0352077
947289	argA	4.33990584	0.0271679
948464	argB	4.1987825	0.0246394
945376	bioA	3.69557079	0.00470518
946932	yffS	3.68202556	1.39E-07
944775	carB	3.64072337	0.00077987
945016	xynR	3.60310199	0.02980869
945388	bioC	3.50785355	0.00185026
947260	rlmC	3.50231116	0.00628941
945387	bioD	3.41356029	0.00250246
946927	yffQ	3.36896256	0.00031234
948463	argH	3.13974762	0.02291143
2847771	ryeA	3.09680575	0.01189623
948448	frwC	2.9711849	0.03614227
946540	flu	2.85048236	0.0004508
946985	guaB	2.76650703	0.00057414
948655	groS	2.68402395	0.01527898
2847691	isrC	2.66304209	0.03449177
947590	argG	2.61966926	0.0246394
945354	galE	2.58651982	1.59E-06
945554	ompF	2.56263927	0.03678747
945357	galT	2.53481482	1.80E-05
947746	gltF	2.4462694	0.00189179
947022	glyA	2.41038212	0.0038078
946237	ydjO	2.38649018	0.00072808
948843	fimC	2.37872494	0.00035226
948665	groL	2.37726146	0.00625887
946544	hisH	2.36882234	0.00051817
947285	suhB	2.32156313	0.02612449
945611	ndk	2.28078217	0.01252996
946551	hisC	2.26288302	0.00051817
947577	rpsU	2.24590386	0.00470518
947231	cysI	2.22139074	0.03593408
946090	cspF	2.20482213	0.03995712

948235	bglG	2.20405136	0.00935343
947547	appB	2.20086813	0.00028236
948838	fimA	2.1932871	0.00280803
945585	appC	2.17511164	0.00031234
948841	fimI	2.16610164	0.0021922
944759	rpsT	2.15713904	0.00368248
948258	asnA	2.15107461	0.0004508
947594	fadH	2.14857376	0.00031234
946531	hisD	2.1417726	0.00073336
944750	dnaK	2.13396004	0.01668446
946889	cysA	2.12669016	0.01127165
946552	hisB	2.12005488	0.00362038
945787	pyrC	2.06501464	0.0048122
2847688	cpxP	2.05556114	0.00075161
948200	ibpA	2.05063845	0.0243004
947582	nfeF	2.03557513	0.02114196
946850	alaC	2.02820972	0.00154412
946521	hisA	2.02791682	0.00136491
948767	pyrB	2.01982375	0.04097363
946975	purM	2.01077147	0.03945266
948723	rpsF	1.98239124	0.01494842
947724	glbB	1.97620611	0.00059041
948912	radA	1.97010953	0.0038078
948722	priB	1.96346383	0.00371158
948244	atpD	1.96241731	0.00152535
946702	mgo	1.95796107	0.00603161
946376	mepM	1.95082741	0.00075161
949031	purE	1.94764918	0.03995712
948251	atpI	1.94041969	0.00208936
945052	yqgC	1.93769688	0.02064975
945099	htpG	1.92828636	0.01214839
948913	serB	1.919514	0.01214839
945859	yciW	1.91703112	0.03923491
945846	trpE	1.90198779	0.00362038
946533	plaP	1.87324388	0.02282638
946705	yojI	1.86975135	0.00944898
948245	atpC	1.86894827	5.59E-05
946882	cysU	1.86704565	0.01655102
948721	rpsR	1.85861206	0.00418964
946516	hisF	1.80788465	0.00625887

947733	yhcC	1.79356805	0.02312118
945772	prs	1.78984337	0.00031234
944994	codB	1.78169148	0.02656349
945864	rnb	1.76854292	0.01213062
947287	recD	1.76046283	0.01232376
947334	guaA	1.74880262	0.00111472
947469	yghF	1.73961482	0.0488145
947499	parC	1.73725473	0.00017055
947457	speC	1.73140214	0.01204684
945462	pdeI	1.72891284	0.01685445
946368	purT	1.72154031	0.01157511
946515	hisI	1.71448118	0.00470518
947420	tktA	1.70966458	0.01690361
945618	rplY	1.69314139	0.00836081
947578	tsaD	1.668928	0.00470518
948243	atpG	1.66480796	0.00497502
948848	gntP	1.64844751	0.02601291
944905	queA	1.62548111	0.00750945
947911	yhgF	1.62074373	0.00488045
946765	mutM	1.62001412	0.01781367
945807	narJ	1.60774901	0.03082372
948845	fimF	1.60573738	0.03449177
947032	purL	1.60338678	0.03995712
948277	ilvD	1.59771005	0.00333899
947723	gltd	1.59111425	0.00221569
948309	bioP	1.58775473	0.01270846
949009	yafT	1.57775867	0.02126244
946565	wcaL	1.57131557	0.00127749
946226	ycgL	1.57012571	0.00050525
947576	ttdT	1.55826348	0.03904529
947924	gntT	1.55347829	0.00040655
945291	ybfE	1.55133018	0.00058833
948482	tufB	1.54788167	0.00377975
947971	livJ	1.54708305	0.03520283
948156	rph	1.53969351	0.02325436
948248	mnmg	1.53039391	0.0051629
946362	edd	1.52007188	0.00058833
947169	pncC	1.51799669	0.01500627
946357	yoaB	1.51610557	0.00241301
949130	tgt	1.50444864	0.00432582

948242	atpA	1.50056652	0.02111879
948720	rplI	1.49761188	0.00630085
945358	galK	1.48926903	0.00466031
947115	tyrA	1.4871142	0.01308436
948844	fimD	1.48381123	0.01878013
944931	yafX	1.47869897	0.02264972
948793	ilvI	1.45224859	0.04805205
944996	codA	1.44125582	0.0071077
948254	atpH	1.43274568	0.01437409
945307	sucB	1.42987876	0.03571625
946206	appA	1.42953975	0.00208936
947806	rplP	1.42580255	0.03055268
947589	rlmG	1.41249822	0.03678747
944753	dnaJ	1.4046323	0.02416433
948316	rhtB	1.40293757	0.00466031
945325	ybgD	1.39770312	0.03172942
947820	rplB	1.39581897	0.03021974
944777	ispH	1.39540877	0.00124507
946998	hcaE	1.389867	0.00470518
945564	rlmL	1.38063522	0.00436401
948103	selB	1.36939995	0.00239391
947758	accB	1.36918969	0.03811525
946794	purF	1.36889433	0.02321125
948247	atpF	1.36521749	0.01500627
948224	adeP	1.35111976	0.02513311
947813	rplV	1.34840503	0.03815493
948157	pyrE	1.34561018	0.04619516
944928	yagF	1.34255263	0.01440851
947234	yegD	1.34213542	0.00762876
949062	yadS	1.34039865	0.00859561
945551	gloC	1.32854934	0.02991473
947814	rpsC	1.32103345	0.0246857
2847752	tatD	1.32041927	0.00200441
947807	rpmC	1.31887655	0.01500627
946888	cysM	1.31435037	0.0271679
945109	trpD	1.30858626	0.02312118
944761	ileS	1.30322256	0.02158327
947808	rpsQ	1.29893341	0.03019833
946267	torA	1.28414829	0.01213062
947049	nadB	1.28316224	0.00320238

948897	prfC	1.28187529	0.01905436
946299	ydjL	1.27915034	0.01788312
948178	uhpA	1.27445573	0.02487785
945768	trpB	1.25201881	0.02622265
948784	yjgN	1.2450638	0.02487785
945549	mukB	1.24266683	0.00312003
947665	yhbP	1.24099924	0.03560219
946924	eutB	1.2406984	0.00614315
948433	metL	1.23729415	0.03904529
947121	pyrF	1.23550407	0.04241239
946512	yeeR	1.23473193	0.011442
948508	yjaH	1.23034781	0.03523588
947907	mrca	1.22139191	0.04368188
947383	aegA	1.21910502	0.01341085
945004	pal	1.21704799	0.00859837
946648	pncB	1.21358097	0.01389997
948287	ilvA	1.20929039	0.00368248
949116	proS	1.20393574	0.02918024
947184	mltB	1.20126251	0.0271679
946690	yejK	1.19439031	0.02746098
948888	opgB	1.1912064	0.02513311
947811	rpsS	1.18140641	0.03530547
948986	guaC	1.17511478	0.02627459
946452	argS	1.1672576	0.02126244
946914	eutL	1.1497864	0.04840471
948766	pepB	1.13898495	0.03593408
946643	metG	1.13472933	0.02068792
945400	yifK	1.13370567	0.0379056
948832	fimB	1.12732483	0.03623362
947294	recC	1.11831675	0.01221591
945539	kdsB	1.11797255	0.01878013
946141	topB	1.11454443	0.03429943
946115	ycgM	1.10585146	0.0052331
945538	ycbK	1.10430259	0.0487748
945496	ybjT	1.06155077	0.03765889
946077	ydhC	1.06101224	0.04471397
944800	lspA	1.0450108	0.02331571
946367	eda	1.03991908	0.04330422
947394	gcvP	1.03474785	0.03788732
946796	accD	1.02435417	0.02868612

945451	folC	1.02006005	0.04093183
945143	lpxM	0.96874184	0.03362508
946160	intA	0.93454218	0.04919768
945863	pgpB	-0.8801531	0.04815468
945448	mdfA	-0.9423186	0.0379056
946772	alaA	-0.9569055	0.0379056
948176	yicL	-0.9713302	0.02312118
947111	abpB	-0.9815761	0.03686383
945858	sohB	-0.9970986	0.03728239
946342	manZ	-1.05173	0.0487748
948155	waaQ	-1.0565355	0.04752972
944840	manA	-1.0695972	0.00651999
947321	clsC	-1.0756213	0.01189623
946332	manY	-1.0854379	0.02064975
945140	yniB	-1.0866964	0.01838547
948861	mdtM	-1.0916628	0.03449177
945304	yccX	-1.0951563	0.0488145
947139	degP	-1.0966744	0.02513311
948230	cbrC	-1.0974567	0.04086619
945935	ybiO	-1.0993247	0.03738517
946801	slyB	-1.102135	0.02873351
947257	qmcA	-1.1025729	0.03593408
946750	elaA	-1.10502	0.02282638
949119	csgD	-1.1085781	0.03945266
948631	basR	-1.1198221	0.03623362
947683	arnA	-1.1205826	0.02550249
947560	mug	-1.1219055	0.04947426
945041	phoA	-1.1261341	0.01266643
945432	ybiV	-1.1308965	0.00762876
948107	yiaU	-1.1519707	0.01883368
945711	csgE	-1.1545629	0.03614227
944783	yabl	-1.1561928	0.03042906
945369	qseB	-1.1581674	0.03923491
945943	ydbL	-1.1611415	0.0418982
945371	mngR	-1.1645024	0.00859561
948050	bcsE	-1.1648017	0.02878904
948632	basS	-1.1658784	0.04471397
949097	fadJ	-1.1671254	0.00601879
946903	yajO	-1.168354	0.01200147
945850	yciQ	-1.1691763	0.03593408

945522	sfmA	-1.1706808	0.04471397
945619	csgG	-1.1776808	0.01422319
947980	yhhT	-1.1780869	0.03082372
946157	leuE	-1.1838964	0.04064219
946740	nudI	-1.2026483	0.02064975
946334	manX	-1.2186486	0.02252426
946421	sdiA	-1.2323253	0.03082372
949055	csgA	-1.2323913	0.00554287
945730	galU	-1.2355236	0.02331571
945988	ycjY	-1.2378528	0.01397106
948775	bdcR	-1.2439141	0.01070416
948822	yfdK	-1.2443331	0.01943807
947005	phr	-1.2479006	0.01314401
945251	ybeQ	-1.252731	0.01413113
945137	fetB	-1.2576313	0.00362038
948755	nrdD	-1.2638971	0.03449177
946253	spy	-1.264282	0.00361978
948728	cysQ	-1.2696065	0.02524871
945622	csgF	-1.2709441	0.01361547
946327	fadD	-1.2870965	0.00165752
945812	fadM	-1.2899612	0.00540533
948561	yjbQ	-1.294404	0.00262615
946131	ynfC	-1.296826	0.02918024
947246	ybdK	-1.299947	0.03571764
947639	prlF	-1.3044623	0.01924838
946130	intQ	-1.3090835	0.04597751
947304	ygdR	-1.3155186	0.04265283
947622	yqjC	-1.3187259	0.03603357
945149	allE	-1.3204004	0.00191097
946679	yejB	-1.334361	0.00279403
945334	arnD	-1.3376084	0.00672743
947176	csrA	-1.3384598	0.02982012
946161	gloA	-1.3439069	0.00470518
948833	tcyJ	-1.3444445	0.00420749
948930	yccJ	-1.3459643	0.01461202
945146	glxR	-1.3599651	0.03581481
947156	ygaC	-1.3670749	0.03449177
947043	bolA	-1.3686849	0.03945266
948033	yhjD	-1.3714131	0.03449177
948540	ubiA	-1.3724258	0.00334529

947495	dkgA	-1.3756232	0.0243004
947222	casA	-1.3777939	0.02126244
945818	oppF	-1.378273	0.02126244
945523	ycaN	-1.378625	0.04337573
945533	ihfB	-1.3797689	0.02416311
946566	yegH	-1.3924584	0.00056546
946990	fetA	-1.3979464	0.00191097
945165	ybcL	-1.4024657	0.01951411
946666	yohC	-1.4034317	0.01039845
945119	fsr	-1.4040071	0.00248207
945860	yciN	-1.4088815	0.00143502
945605	aroG	-1.4092743	0.02991473
948680	frdC	-1.4299177	0.03904529
947615	yqjG	-1.4302279	0.02068792
945889	ompG	-1.43129	0.04910009
946486	hprR	-1.4330515	0.00562916
945472	ihfA	-1.4449699	0.011442
945273	ldtA	-1.4472913	0.02758249
946029	narV	-1.4544367	0.011442
948566	soxR	-1.4602085	0.00965438
2847763	yedS	-1.4619136	0.00991513
947356	mocA	-1.4720351	0.011442
944837	yadC	-1.4725223	0.01422319
945401	ybhL	-1.4731427	0.00466031
945909	dgcM	-1.4764914	0.00368248
947165	proX	-1.4787495	0.03563346
948647	yjdJ	-1.4811484	0.03263271
948730	ytfH	-1.4828942	0.04264037
944902	gloB	-1.4939608	0.00134539
946075	dgcZ	-1.4992312	0.00147437
947532	hemH	-1.5078483	0.00836081
946825	tfaS	-1.5095355	0.0271679
948622	yjdN	-1.5120101	0.01437409
949118	gfcB	-1.5122607	0.014586
948621	yjdM	-1.5141447	0.00470518
946406	otsB	-1.5246273	0.02918024
948568	pdeC	-1.5404778	0.00147437
945982	chbC	-1.5446784	0.00935343
947297	arnT	-1.5447226	0.00334529
948777	tabA	-1.5465121	0.00074985

948077	wecH	-1.5549505	0.00129243
945905	ykgJ	-1.5583042	0.01525798
946168	ydhL	-1.5631147	0.04141167
946987	ymdB	-1.5678954	0.00466031
945160	sfmD	-1.5791012	0.02815944
947144	kbp	-1.5822306	0.03496198
946546	sbmC	-1.5864947	0.01899081
944828	yadM	-1.5875526	0.03678747
946308	yoaC	-1.5881211	0.01158148
948175	nlpA	-1.5957175	0.00075161
948037	treF	-1.5977974	0.00859561
945868	pdeR	-1.6021637	0.00720069
4056028	yncK	-1.6037521	0.00070577
946059	pqqL	-1.6072504	0.03811525
945724	tfaP	-1.6246135	0.02991473
948051	yhjR	-1.6261282	0.01437409
944835	yadK	-1.6367993	0.00470518
948141	xylA	-1.6619762	0.00058833
946365	ycgB	-1.6710622	0.00368248
947956	yhhA	-1.6736912	0.02007098
947225	casD	-1.6743505	0.00143502
946435	yedK	-1.6865376	0.02344064
947095	yegP	-1.7009322	0.00379558
945623	csgC	-1.7056943	0.01761705
945830	oppA	-1.7058179	0.00179133
946681	osmF	-1.7072287	0.01221591
946043	osmC	-1.7168406	0.011442
948078	yiaA	-1.7232243	0.011442
945829	hns	-1.727914	0.00051817
944829	yadL	-1.7292681	0.00191097
945677	yagU	-1.7294645	0.00059041
944956	ykgA	-1.7310289	0.00011284
945802	oppD	-1.7379801	0.00415775
945786	ydhS	-1.7412577	0.00232945
948058	bcsG	-1.7441534	0.00031234
2847756	cutC	-1.7486138	0.0322881
945976	ydcU	-1.7573331	1.78E-05
947114	rcnR	-1.7594438	0.0246857
949020	idi	-1.7600588	0.00051817
946436	ydgD	-1.771093	0.00121083

946259	astB	-1.7770947	0.04314698
949012	ymdA	-1.7991975	0.02325436
947201	yegJ	-1.8077856	0.00467324
945308	yaiP	-1.8108881	0.00558592
945101	dps	-1.8252623	0.03218899
946007	ydcT	-1.8510662	4.81E-05
945903	ydcV	-1.858066	0.00028618
947353	glcD	-1.8729123	0.02282638
945815	dosP	-1.8855082	0.00075161
947451	ydfK	-1.8960475	0.0246394
948737	ytfJ	-1.8985931	5.54E-06
948090	xylF	-1.9252532	0.00982612
946172	ydgA	-1.9301643	0.00045015
945805	ygiV	-1.9364665	0.00507568
947224	casC	-1.9401146	0.00051817
948087	avtA	-1.9460821	5.59E-05
946032	narW	-1.962373	0.03765889
945946	ynbE	-1.9694585	0.01500627
947787	mscL	-1.9778668	0.03995712
946151	hdhA	-2.0028959	0.00466031
946485	yedX	-2.0143336	1.78E-05
1450300	yicS	-2.026298	0.0076524
947392	flxA	-2.0307723	0.00139916
945810	oppC	-2.0568877	0.00154412
946432	yfdE	-2.0807329	0.00185026
944968	yahN	-2.0931215	0.00603161
947391	csgB	-2.1175989	0.00147437
945028	yqeG	-2.1185403	0.00168087
946005	ydcS	-2.1229551	0.00031234
946092	rclA	-2.1400034	0.00036168
945305	osmE	-2.1522684	0.01213062
945493	ybjQ	-2.1673913	0.00200441
945491	ybjP	-2.1972128	0.01710802
948076	csiD	-2.1972862	0.04559072
946843	nanC	-2.1997842	0.00269468
948377	yihO	-2.2082924	5.59E-05
946255	astC	-2.2127111	0.03945266
947157	ygaM	-2.2201169	0.00442083
947226	casE	-2.2231331	0.00051817
948203	ydcL	-2.2816808	0.0001994

946051	ygiW	-2.2832943	0.00968638
947959	ugpE	-2.2880331	0.01905436
945823	oppB	-2.3259668	0.00072808
945252	ybeL	-2.336145	0.0019531
946056	ppiD	-2.3424046	2.41E-06
946311	yeaQ	-2.3488621	0.03945266
946034	narY	-2.356959	0.00139916
945835	dosC	-2.3660768	0.00045015
948863	ybaA	-2.3798529	0.00092751
945999	narZ	-2.3878317	0.0019551
946067	ydeJ	-2.391633	0.00051817
947375	arnB	-2.4387805	0.00058833
944853	ybgA	-2.4427718	0.00470518
945723	tfaE	-2.4457698	0.00037072
945275	arnC	-2.4491191	0.00058833
947138	yqaE	-2.4667151	0.03631127
947148	proV	-2.5254245	0.00051817
948630	ypjA	-2.5434198	0.00037358
947835	yhcN	-2.6224121	0.00311249
948895	osmY	-2.6524395	0.01708963
948835	nanS	-2.7441181	0.00012438
946068	ydeI	-2.8604768	0.00057414
944985	yahO	-2.8734525	0.00362038
946287	puuP	-2.8831205	0.00058833
945886	puuR	-2.9188162	0.00058833
1450298	ytjA	-2.9364888	0.01461202
947276	ygdI	-3.0298028	9.66E-06
945446	puuE	-3.1643647	0.00208395
945356	ybgS	-3.2909541	0.00079402
948553	yjbJ	-3.3132296	0.00859837
949106	nanM	-3.6923437	1.05E-06
947003	puuC	-3.7580146	0.00615648
945799	narU	-3.8144767	0.00010656
945072	puuB	-3.8570711	0.00299905
946202	puuA	-3.9841663	0.00056546
945882	puuD	-4.174614	0.00240608
948499	hupA	-11.012093	1.41E-13
949095	hupB	-14.488515	1.59E-06

Table S3. Differentially expressed genes in the strain expressing HU α_2 P63A relative to the strain expressing wild type HU α_2

Entrez			
Gene ID	Symbol	logFC	FDR
945384	bioF	4.55823967	0.00762931
945388	bioC	3.8080242	0.00391923
945370	bioB	3.79069704	0.01739919
949025	carA	3.70655148	0.04888501
945554	ompF	3.48054998	0.01489973
2847771	ryeA	3.45260173	0.01476356
945376	bioA	3.34127659	0.02069611
944775	carB	3.30624762	0.00579353
946932	yffS	3.25075944	6.38E-06
945387	bioD	3.24876843	0.0080857
948235	bglG	3.04156406	0.00473918
947965	livH	2.96643617	0.04480328
944928	yagF	2.9052264	3.33E-05
947285	suhB	2.77778855	0.02138806
947746	glfF	2.70402594	0.00358214
946985	guaB	2.69661517	0.00358214
944994	codB	2.64650699	0.00537473
948841	fimI	2.6172324	0.00322074
946702	mgo	2.58722773	0.00327148
947022	glyA	2.57448756	0.00752906
945426	ybiA	2.56563019	0.04888501
948843	fimC	2.52872856	0.00123905
945357	galT	2.50136207	0.00016442
947231	cysI	2.48926298	0.03733194
948838	fimA	2.40691068	0.00529257
945354	galE	2.40288389	3.33E-05
946544	hisH	2.31984529	0.00327148
948665	groL	2.31770231	0.01947779
948844	fimD	2.28711899	0.00327148
947594	fadH	2.27542735	0.00123905
946786	nmpC	2.27345008	0.01662143
946521	hisA	2.26861938	0.00327148
946533	plaP	2.21827444	0.01754936
944759	rpsT	2.20785916	0.00912709

948912	radA	2.20287551	0.00579353
948251	atpI	2.19943418	0.00391923
948913	serB	2.18950879	0.01286836
946889	cysA	2.18078021	0.02110759
948244	atpD	2.16490403	0.00358214
948767	pyrB	2.15972594	0.04539262
945325	ybgD	2.15114331	0.00489369
946292	ydjI	2.13845402	0.04545671
945611	ndk	2.12177875	0.03999357
944925	yagE	2.11847074	0.00327148
946551	hisC	2.09370004	0.00478787
944905	queA	2.09174241	0.003974
945462	pdeI	2.04297746	0.01450074
948846	fimG	2.02734557	0.03042434
946516	hisF	2.01232186	0.00840925
945329	ymfD	1.99776684	0.00327148
945307	sucB	1.97911296	0.00992132
946237	ydjO	1.96817874	0.01134374
948243	atpG	1.9668509	0.00529257
946552	hisB	1.93720983	0.01781514
946239	ynjI	1.92027012	0.00711958
946531	hisD	1.90625302	0.00711958
947420	tktA	1.89459794	0.02069611
945312	sucC	1.88906992	0.02478982
945787	pyrC	1.88829899	0.02212581
948722	priB	1.88644588	0.01461053
947971	livJ	1.8789906	0.02315359
947334	guaA	1.87882305	0.00327148
945052	yqgC	1.87622531	0.04603107
947818	rplD	1.87145456	0.01391557
947115	tyrA	1.85786255	0.00666968
947457	speC	1.84054343	0.01947779
948254	atpH	1.83557747	0.00579353
947819	rplW	1.83435017	0.00765908
946515	hisI	1.83006721	0.00880217
947820	rplB	1.82837997	0.01134374
945864	rnb	1.82475081	0.02251467
947817	rplC	1.82212007	0.02307344
946979	upp	1.82191458	0.01489973
947032	purL	1.82175038	0.04197073

947924	gntT	1.82024282	0.00039207
949130	tgt	1.81699181	0.00358214
946285	ydjH	1.81109417	0.01391557
947724	gltB	1.79503356	0.00579353
946850	alaC	1.78996881	0.01322558
948793	ilvI	1.78614303	0.03056207
948258	asnA	1.78026997	0.00768013
948224	adeP	1.76865246	0.0080857
948242	atpA	1.75946919	0.01641623
948721	rpsR	1.75485811	0.01703671
946882	cysU	1.75479176	0.0472252
947099	trmD	1.74827358	0.02520373
948156	rph	1.74039846	0.02251467
946705	yojI	1.72734464	0.03469231
946376	mepM	1.72244681	0.00820884
947814	rpsC	1.71747698	0.0080857
948247	atpF	1.71495911	0.00666968
944996	codA	1.69563964	0.00579353
945314	sucD	1.69478509	0.01666593
947499	parC	1.68278498	0.00176629
947911	yhgF	1.66825727	0.01218447
947806	rpIP	1.66545654	0.02363615
945358	galK	1.66289535	0.00579353
949009	yafT	1.65083813	0.03640773
948309	bioP	1.64640003	0.02399005
945556	pyrD	1.64125204	0.03898697
948963	ygiQ	1.61358608	0.04711142
945303	sucA	1.60159519	0.03056207
947723	gltD	1.59782361	0.00765908
945803	thrA	1.59777949	0.04539262
947811	rpsS	1.58957412	0.00782917
947758	accB	1.5879646	0.03200594
947333	mdtI	1.57745448	0.03932562
945213	cstA	1.57463811	0.00565828
947234	yegD	1.57342195	0.00579353
944931	yafX	1.57117791	0.03374068
945772	prs	1.56149354	0.00529257
947813	rpIV	1.55370051	0.0333304
948482	tufB	1.53752417	0.01286836
944753	dnaJ	1.53465974	0.02812595

948252	atpB	1.5240011	0.01776614
945097	adk	1.52009703	0.0284626
944761	ileS	1.51169924	0.01641623
948216	rpmH	1.51011866	0.04603107
947169	pncC	1.50837566	0.03469231
948245	atpC	1.49337993	0.00473918
945729	iraM	1.4641281	0.03550052
947047	yfiC	1.45866018	0.04432587
946923	intZ	1.45597499	0.0376734
944938	rlmF	1.4460514	0.03824481
948483	rpIA	1.44104748	0.03609688
948277	ilvD	1.43989279	0.02195207
946693	lpxT	1.43968338	0.03224284
946888	cysM	1.42188272	0.03374068
948248	mnmg	1.41889485	0.02520373
948784	yjgN	1.41813026	0.02098166
945602	putP	1.41362081	0.0268539
948897	prfC	1.41011799	0.02069611
944824	mutT	1.39035112	0.03195529
947907	mrcA	1.37188113	0.04235929
947804	rpIR	1.34771543	0.03385372
947802	rpsH	1.32665646	0.04571224
946357	yoaB	1.31890644	0.02399005
949116	proS	1.30918378	0.03374068
946362	edd	1.30232231	0.01200613
948720	rpII	1.29295445	0.04888501
947286	recB	1.27593441	0.04493055
945400	yifK	1.26965781	0.03385372
949062	yadS	1.26027191	0.0347192
945006	lacZ	1.22740234	0.03507773
944800	lspA	1.22110872	0.01314202
945564	rlmL	1.17697681	0.04539262
944777	ispH	1.15301238	0.0270319
945004	pal	1.13643102	0.03898697
945549	mukB	1.11043887	0.0284626
946614	gyrA	1.09963916	0.04605324
944840	manA	-0.9887372	0.04352975
948737	ytfJ	-1.0530501	0.04380073
947812	degQ	-1.0851471	0.03550052
948864	dnaC	-1.1233707	0.03511566

946566	yegH	-1.1304016	0.02283447
948561	yjbQ	-1.1332999	0.0333304
945812	fadM	-1.1647457	0.04352975
945860	yciN	-1.178247	0.03056207
944902	gloB	-1.1869332	0.03941223
946315	ldhA	-1.1982661	0.02069611
945858	sohB	-1.2013658	0.01781514
948568	pdeC	-1.2442819	0.03682206
948141	xylA	-1.2568668	0.03385372
947005	phr	-1.2663276	0.03195529
945334	arnD	-1.2700733	0.0347192
948671	gdx	-1.2706152	0.01592479
948058	bcsG	-1.2749221	0.02478982
946485	yedX	-1.2776388	0.01666593
945976	ydcU	-1.2870675	0.00711958
945830	oppA	-1.3338162	0.04539262
945903	ydcV	-1.3412799	0.02288042
945472	ihfA	-1.3617798	0.04605324
948055	csiR	-1.3637507	0.04380073
946409	araF	-1.3734794	0.03511566
947550	ybgO	-1.3739826	0.04603107
946296	yeaH	-1.4161227	0.02110759
946253	spy	-1.426683	0.00529257
948930	yccJ	-1.4582948	0.02283447
4056028	ynck	-1.4972417	0.00774761
944956	ykgA	-1.502308	0.00529257
948077	wecH	-1.5035615	0.00955245
946297	yeaG	-1.5047838	0.02195207
945401	ybhL	-1.5081423	0.01489973
948728	cysQ	-1.5111654	0.02019293
947263	wrbA	-1.5148344	0.04235929
946308	yoaC	-1.5157426	0.04545671
947622	yqjC	-1.5232716	0.03678949
946172	ydgA	-1.5338096	0.01509743
948757	nrdG	-1.5486995	0.01489973
945644	ydjM	-1.5560002	0.02019293
948755	nrdD	-1.5562678	0.02195207
945988	ycjY	-1.5610765	0.00666968
945533	ihfB	-1.6055777	0.02363615
946007	ydcT	-1.612442	0.00327148

946546	sbmC	-1.6246463	0.04585618
947246	ybdK	-1.6296175	0.02179444
947495	dkgA	-1.633728	0.02165201
1450233	yaeP	-1.6399119	0.02195207
948033	yhjD	-1.6717653	0.02666648
946406	otsB	-1.6995764	0.04352975
947381	yqfA	-1.702105	0.03068966
948630	ypjA	-1.7256151	0.03195529
949118	gfcB	-1.752719	0.02507377
946953	yffB	-1.7545927	0.01489973
946005	ydcS	-1.7807252	0.00774761
945786	ydhS	-1.7887209	0.00912709
947615	yqjG	-1.8082251	0.01391557
947095	yegP	-1.8131504	0.01113369
946436	ydgD	-1.813296	0.00579353
948071	yiaG	-1.8334938	0.03971149
947956	yhhA	-1.8487306	0.0347383
944932	ydcK	-1.8689738	0.02165201
948087	avtA	-1.8952843	0.00123905
946151	hdhA	-1.9111545	0.02516597
945835	dosC	-1.9189969	0.0127056
945275	arnC	-1.9305001	0.02110759
946067	ydeJ	-1.9452395	0.01489973
945623	csgC	-1.964753	0.0455751
948037	treF	-1.9870771	0.00771219
948203	ydcL	-1.9992656	0.00500229
946481	hchA	-2.022556	0.02195207
945493	ybjQ	-2.0361602	0.01641623
946365	ycgB	-2.0437287	0.00529257
946092	rclA	-2.0452063	0.00473918
945805	ygiV	-2.0568077	0.01641623
948863	ybaA	-2.0709113	0.01489973
947157	ygaM	-2.1056624	0.02488548
947451	ydfK	-2.1167655	0.03932562
946043	osmC	-2.1381863	0.01098774
945305	osmE	-2.2000013	0.03507773
945905	ykgJ	-2.2084995	0.00812013
946259	astB	-2.2660532	0.03609688
948835	nanS	-2.2693218	0.00711958
947375	arnB	-2.2717701	0.00765817

945252	ybeL	-2.3055035	0.01175065
946435	yedK	-2.3189939	0.01457156
947276	ygdI	-2.3355548	0.00322074
944853	ybgA	-2.3630612	0.02432148
946418	yfcS	-2.4552414	0.01641623
946068	ydeI	-2.5530782	0.00848888
946256	astE	-2.5550743	0.01710935
946051	ygiW	-2.5708449	0.01781514
946260	astD	-2.6844739	0.02363615
948895	osmY	-2.7065131	0.04888501
949106	nanM	-2.7240918	0.00097441
947835	yhcN	-2.7790572	0.01115934
945886	puuR	-2.8534002	0.00579353
944985	yahO	-2.9619996	0.01489973
945101	dps	-3.0325999	0.00579353
945799	narU	-3.0404384	0.00579353
1450298	ytjA	-3.1113093	0.03898697
946255	astC	-3.257092	0.01914093
946287	puuP	-3.3737961	0.00322074
945356	ybgS	-3.416793	0.00574647
946261	astA	-3.6534705	0.02212581
945446	puuE	-3.7505989	0.00574647
948553	yjbJ	-3.908921	0.01641623
946202	puuA	-3.9668766	0.00529257
945072	puuB	-4.2654069	0.01083335
947003	puuC	-4.2726568	0.01646859
945882	puuD	-4.3694047	0.01175065

Table S4 Overlap of differentially expressed genes between the HU null strain (relative to the strain expressing wild type *hup* genes) and the strain expressing HU α_2 P63A (relative to the strain expressing wild type HU α_2)

HU null vs WT only

Gene	logFC	Trend
pgpB	-0.8801531	DOWN
mdfA	-0.9423186	DOWN
alaA	-0.9569055	DOWN
yicL	-0.9713302	DOWN

abpB	-0.9815761	DOWN
manZ	-1.05173	DOWN
waaQ	-1.0565355	DOWN
clsC	-1.0756213	DOWN
manY	-1.0854379	DOWN
yniB	-1.0866964	DOWN
mdtM	-1.0916628	DOWN
yccX	-1.0951563	DOWN
degP	-1.0966744	DOWN
cbrC	-1.0974567	DOWN
ybiO	-1.0993247	DOWN
slyB	-1.102135	DOWN
qmcA	-1.1025729	DOWN
elaA	-1.10502	DOWN
csgD	-1.1085781	DOWN
basR	-1.1198221	DOWN
arnA	-1.1205826	DOWN
mug	-1.1219055	DOWN
phoA	-1.1261341	DOWN
ybiV	-1.1308965	DOWN
yaU	-1.1519707	DOWN
csgE	-1.1545629	DOWN
yabI	-1.1561928	DOWN
qseB	-1.1581674	DOWN
ydbL	-1.1611415	DOWN
mngR	-1.1645024	DOWN
bcsE	-1.1648017	DOWN
basS	-1.1658784	DOWN
fadJ	-1.1671254	DOWN
yajO	-1.168354	DOWN
yciQ	-1.1691763	DOWN
sfmA	-1.1706808	DOWN
csgG	-1.1776808	DOWN
yhhT	-1.1780869	DOWN
leuE	-1.1838964	DOWN
nudI	-1.2026483	DOWN
manX	-1.2186486	DOWN
sdiA	-1.2323253	DOWN
csgA	-1.2323913	DOWN
galU	-1.2355236	DOWN

bdcR	-1.2439141	DOWN
yfdK	-1.2443331	DOWN
ybeQ	-1.252731	DOWN
fetB	-1.2576313	DOWN
csgF	-1.2709441	DOWN
fadD	-1.2870965	DOWN
ynfC	-1.296826	DOWN
prlF	-1.3044623	DOWN
intQ	-1.3090835	DOWN
ygdR	-1.3155186	DOWN
allE	-1.3204004	DOWN
yejB	-1.334361	DOWN
csrA	-1.3384598	DOWN
gloA	-1.3439069	DOWN
tcyJ	-1.344445	DOWN
glxR	-1.3599651	DOWN
ygaC	-1.3670749	DOWN
bolA	-1.3686849	DOWN
ubiA	-1.3724258	DOWN
casA	-1.3777939	DOWN
oppF	-1.378273	DOWN
ycaN	-1.378625	DOWN
fetA	-1.3979464	DOWN
ybcL	-1.4024657	DOWN
yohC	-1.4034317	DOWN
fsr	-1.4040071	DOWN
aroG	-1.4092743	DOWN
frdC	-1.4299177	DOWN
ompG	-1.43129	DOWN
hprR	-1.4330515	DOWN
ldtA	-1.4472913	DOWN
narV	-1.4544367	DOWN
soxR	-1.4602085	DOWN
yedS	-1.4619136	DOWN
mocA	-1.4720351	DOWN
yadC	-1.4725223	DOWN
dgcM	-1.4764914	DOWN
proX	-1.4787495	DOWN
yjdJ	-1.4811484	DOWN
ytfH	-1.4828942	DOWN

dgcZ	-1.4992312	DOWN
hemH	-1.5078483	DOWN
tfaS	-1.5095355	DOWN
yjdN	-1.5120101	DOWN
yjdM	-1.5141447	DOWN
chbC	-1.5446784	DOWN
arnT	-1.5447226	DOWN
tabA	-1.5465121	DOWN
ydhL	-1.5631147	DOWN
ymdB	-1.5678954	DOWN
sfmD	-1.5791012	DOWN
kbp	-1.5822306	DOWN
yadM	-1.5875526	DOWN
nlpA	-1.5957175	DOWN
pdeR	-1.6021637	DOWN
pqqL	-1.6072504	DOWN
tfaP	-1.6246135	DOWN
yhjR	-1.6261282	DOWN
yadK	-1.6367993	DOWN
casD	-1.6743505	DOWN
osmF	-1.7072287	DOWN
yiaA	-1.7232243	DOWN
hns	-1.727914	DOWN
yadL	-1.7292681	DOWN
yagU	-1.7294645	DOWN
oppD	-1.7379801	DOWN
cutC	-1.7486138	DOWN
rcnR	-1.7594438	DOWN
idi	-1.7600588	DOWN
ymdA	-1.7991975	DOWN
yegJ	-1.8077856	DOWN
yaiP	-1.8108881	DOWN
glcD	-1.8729123	DOWN
dosP	-1.8855082	DOWN
xylF	-1.9252532	DOWN
casC	-1.9401146	DOWN
narW	-1.962373	DOWN
ynbE	-1.9694585	DOWN
mscL	-1.9778668	DOWN
yicS	-2.026298	DOWN

flxA	-2.0307723	DOWN
oppC	-2.0568877	DOWN
yfdE	-2.0807329	DOWN
yahN	-2.0931215	DOWN
csgB	-2.1175989	DOWN
yqeG	-2.1185403	DOWN
ybjP	-2.1972128	DOWN
csiD	-2.1972862	DOWN
nanC	-2.1997842	DOWN
yihO	-2.2082924	DOWN
casE	-2.2231331	DOWN
ugpE	-2.2880331	DOWN
oppB	-2.3259668	DOWN
ppiD	-2.3424046	DOWN
yeaQ	-2.3488621	DOWN
narY	-2.356959	DOWN
narZ	-2.3878317	DOWN
tfaE	-2.4457698	DOWN
yqaE	-2.4667151	DOWN
proV	-2.5254245	DOWN
hupA	-11.012093	DOWN
hupB	-14.488515	DOWN
argF	5.5605087	UP
argI	4.8558759	UP
argC	4.4221709	UP
artJ	4.4151406	UP
argA	4.3399058	UP
argB	4.1987825	UP
xynR	3.603102	UP
rlmC	3.5023112	UP
yffQ	3.3689626	UP
argH	3.1397476	UP
frwC	2.9711849	UP
flu	2.8504824	UP
groS	2.6840239	UP
isrC	2.6630421	UP
argG	2.6196693	UP
rpsU	2.2459039	UP
cspF	2.2048221	UP
appB	2.2008681	UP

appC	2.1751116	UP
dnaK	2.13396	UP
cpxP	2.0555611	UP
ibpA	2.0506384	UP
nfeF	2.0355751	UP
purM	2.0107715	UP
rpsF	1.9823912	UP
purE	1.9476492	UP
htpG	1.9282864	UP
yciW	1.9170311	UP
trpE	1.9019878	UP
yhcC	1.793568	UP
recD	1.7604628	UP
yghF	1.7396148	UP
purT	1.7215403	UP
rplY	1.6931414	UP
tsaD	1.668928	UP
gntP	1.6484475	UP
mutM	1.6200141	UP
narJ	1.607749	UP
fimF	1.6057374	UP
wcaL	1.5713156	UP
ycgL	1.5701257	UP
ttdT	1.5582635	UP
ybfE	1.5513302	UP
appA	1.4295397	UP
rlmG	1.4124982	UP
rhtB	1.4029376	UP
hcaE	1.389867	UP
selB	1.3694	UP
purF	1.3688943	UP
pyrE	1.3456102	UP
gloC	1.3285493	UP
tatD	1.3204193	UP
rpmC	1.3188765	UP
trpD	1.3085863	UP
rpsQ	1.2989334	UP
torA	1.2841483	UP
nadB	1.2831622	UP
ydjL	1.2791503	UP

uhpA	1.2744557	UP
trpB	1.2520188	UP
yhbP	1.2409992	UP
eutB	1.2406984	UP
metL	1.2372941	UP
pyrF	1.2355041	UP
yeeR	1.2347319	UP
yjaH	1.2303478	UP
aegA	1.219105	UP
pncB	1.213581	UP
ilvA	1.2092904	UP
mltB	1.2012625	UP
yejK	1.1943903	UP
opgB	1.1912064	UP
guaC	1.1751148	UP
argS	1.1672576	UP
eutL	1.1497864	UP
pepB	1.1389849	UP
metG	1.1347293	UP
fimB	1.1273248	UP
recC	1.1183167	UP
kdsB	1.1179725	UP
topB	1.1145444	UP
ycgM	1.1058515	UP
ycbK	1.1043026	UP
ybjT	1.0615508	UP
ydhC	1.0610122	UP
eda	1.0399191	UP
gcvP	1.0347478	UP
accD	1.0243542	UP
folC	1.0200601	UP
lpxM	0.9687418	UP
intA	0.9345422	UP

HU α_2 P63A vs wt HU α_2 only

	logFC	Trend
degQ	-1.085147	DOWN
dnaC	-1.123371	DOWN
ldhA	-1.198266	DOWN
gdx	-1.270615	DOWN

csiR	-1.363751	DOWN
araF	-1.373479	DOWN
ybgO	-1.373983	DOWN
yeaH	-1.416123	DOWN
yeaG	-1.504784	DOWN
wrbA	-1.514834	DOWN
nrdG	-1.548699	DOWN
ydjM	-1.556	DOWN
yaeP	-1.639912	DOWN
yqfA	-1.702105	DOWN
yffB	-1.754593	DOWN
yiaG	-1.833494	DOWN
ydcK	-1.868974	DOWN
hchA	-2.022556	DOWN
yfcS	-2.455241	DOWN
astE	-2.555074	DOWN
astD	-2.684474	DOWN
astA	-3.653471	DOWN
livH	2.966436	UP
ybiA	2.56563	UP
nmpC	2.27345	UP
ydjJ	2.138454	UP
yagE	2.118471	UP
fimG	2.027346	UP
ymfD	1.997767	UP
ynjI	1.92027	UP
sucC	1.88907	UP
rplD	1.871455	UP
rplW	1.83435	UP
rplC	1.82212	UP
upp	1.821915	UP
ydjH	1.811094	UP
trmD	1.748274	UP
sucD	1.694785	UP
pyrD	1.641252	UP
ygiQ	1.613586	UP
sucA	1.601595	UP
thrA	1.597779	UP
mdtI	1.577454	UP
cstA	1.574638	UP

atpB	1.524001	UP
adk	1.520097	UP
rpmH	1.510119	UP
iraM	1.464128	UP
yfiC	1.45866	UP
intZ	1.455975	UP
rlmF	1.446051	UP
rplA	1.441047	UP
lpxT	1.439683	UP
putP	1.413621	UP
mutT	1.390351	UP
rplR	1.347715	UP
rpsH	1.326656	UP
recB	1.275934	UP
lacZ	1.227402	UP
gyrA	1.099639	UP

Common DEGs in HU null vs WT (A) and HU α_2 P63A vs wt HU α_2 (B)

	logFC (A)	logFC (B)	Trend
arnB	-2.4387805	-2.2717701	DOWN
arnC	-2.4491191	-1.9305001	DOWN
arnD	-1.3376084	-1.2700733	DOWN
astB	-1.7770947	-2.2660532	DOWN
astC	-2.2127111	-3.257092	DOWN
avtA	-1.9460821	-1.8952843	DOWN
bcsG	-1.7441534	-1.2749221	DOWN
csgC	-1.7056943	-1.964753	DOWN
cysQ	-1.2696065	-1.5111654	DOWN
dkgA	-1.3756232	-1.633728	DOWN
dosC	-2.3660768	-1.9189969	DOWN
dps	-1.8252623	-3.0325999	DOWN
fadM	-1.2899612	-1.1647457	DOWN
gfcB	-1.5122607	-1.752719	DOWN
gloB	-1.4939608	-1.1869332	DOWN
hdhA	-2.0028959	-1.9111545	DOWN
ihfA	-1.4449699	-1.3617798	DOWN
ihfB	-1.3797689	-1.6055777	DOWN
manA	-1.0695972	-0.9887372	DOWN
nanM	-3.6923437	-2.7240918	DOWN

nanS	-2.7441181	-2.2693218	DOWN
narU	-3.8144767	-3.0404384	DOWN
nrdD	-1.2638971	-1.5562678	DOWN
oppA	-1.7058179	-1.3338162	DOWN
osmC	-1.7168406	-2.1381863	DOWN
osmE	-2.1522684	-2.2000013	DOWN
osmY	-2.6524395	-2.7065131	DOWN
otsB	-1.5246273	-1.6995764	DOWN
pdeC	-1.5404778	-1.2442819	DOWN
phr	-1.2479006	-1.2663276	DOWN
puuA	-3.9841663	-3.9668766	DOWN
puuB	-3.8570711	-4.2654069	DOWN
puuC	-3.7580146	-4.2726568	DOWN
puuD	-4.174614	-4.3694047	DOWN
puuE	-3.1643647	-3.7505989	DOWN
puuP	-2.8831205	-3.3737961	DOWN
puuR	-2.9188162	-2.8534002	DOWN
rclA	-2.1400034	-2.0452063	DOWN
sbmC	-1.5864947	-1.6246463	DOWN
sohB	-0.9970986	-1.2013658	DOWN
spy	-1.264282	-1.426683	DOWN
treF	-1.5977974	-1.9870771	DOWN
wecH	-1.5549505	-1.5035615	DOWN
xylA	-1.6619762	-1.2568668	DOWN
yahO	-2.8734525	-2.9619996	DOWN
ybaA	-2.3798529	-2.0709113	DOWN
ybdK	-1.299947	-1.6296175	DOWN
ybeL	-2.336145	-2.3055035	DOWN
ybgA	-2.4427718	-2.3630612	DOWN
ybgS	-3.2909541	-3.416793	DOWN
ybhL	-1.4731427	-1.5081423	DOWN
ybjQ	-2.1673913	-2.0361602	DOWN
yccJ	-1.3459643	-1.4582948	DOWN
ycgB	-1.6710622	-2.0437287	DOWN
yciN	-1.4088815	-1.178247	DOWN
ycjY	-1.2378528	-1.5610765	DOWN
ydcL	-2.2816808	-1.9992656	DOWN
ydcS	-2.1229551	-1.7807252	DOWN
ydcT	-1.8510662	-1.612442	DOWN
ydcU	-1.7573331	-1.2870675	DOWN

ydcV	-1.858066	-1.3412799	DOWN
ydeI	-2.8604768	-2.5530782	DOWN
ydeJ	-2.391633	-1.9452395	DOWN
ydfK	-1.8960475	-2.1167655	DOWN
ydgA	-1.9301643	-1.5338096	DOWN
ydgD	-1.771093	-1.813296	DOWN
ydhS	-1.7412577	-1.7887209	DOWN
yedK	-1.6865376	-2.3189939	DOWN
yedX	-2.0143336	-1.2776388	DOWN
yegH	-1.3924584	-1.1304016	DOWN
yegP	-1.7009322	-1.8131504	DOWN
ygaM	-2.2201169	-2.1056624	DOWN
ygdI	-3.0298028	-2.3355548	DOWN
ygiV	-1.9364665	-2.0568077	DOWN
ygiW	-2.2832943	-2.5708449	DOWN
yhcN	-2.6224121	-2.7790572	DOWN
yhhA	-1.6736912	-1.8487306	DOWN
yhjD	-1.3714131	-1.6717653	DOWN
yjbJ	-3.3132296	-3.908921	DOWN
yjbQ	-1.294404	-1.1332999	DOWN
ykgA	-1.7310289	-1.502308	DOWN
ykgJ	-1.5583042	-2.2084995	DOWN
yncK	-1.6037521	-1.4972417	DOWN
yoaC	-1.5881211	-1.5157426	DOWN
ypjA	-2.5434198	-1.7256151	DOWN
yqjC	-1.3187259	-1.5232716	DOWN
yqjG	-1.4302279	-1.8082251	DOWN
ytfJ	-1.8985931	-1.0530501	DOWN
ytjA	-2.9364888	-3.1113093	DOWN
accB	1.3691897	1.5879646	UP
adeP	1.3511198	1.7686525	UP
alaC	2.0282097	1.7899688	UP
asnA	2.1510746	1.78027	UP
atpA	1.5005665	1.7594692	UP
atpC	1.8689483	1.4933799	UP
atpD	1.9624173	2.164904	UP
atpF	1.3652175	1.7149591	UP
atpG	1.664808	1.9668509	UP
atpH	1.4327457	1.8355775	UP
atpI	1.9404197	2.1994342	UP

bglG	2.2040514	3.0415641	UP
bioA	3.6955708	3.3412766	UP
bioB	4.5907029	3.790697	UP
bioC	3.5078535	3.8080242	UP
bioD	3.4135603	3.2487684	UP
bioF	4.5446014	4.5582397	UP
bioP	1.5877547	1.6464	UP
carA	5.2953578	3.7065515	UP
carB	3.6407234	3.3062476	UP
codA	1.4412558	1.6956396	UP
codB	1.7816915	2.646507	UP
cysA	2.1266902	2.1807802	UP
cysI	2.2213907	2.489263	UP
cysM	1.3143504	1.4218827	UP
cysU	1.8670456	1.7547918	UP
dnaJ	1.4046323	1.5346597	UP
edd	1.5200719	1.3023223	UP
fadH	2.1485738	2.2754274	UP
fimA	2.1932871	2.4069107	UP
fimC	2.3787249	2.5287286	UP
fimD	1.4838112	2.287119	UP
fimI	2.1661016	2.6172324	UP
galE	2.5865198	2.4028839	UP
galK	1.489269	1.6628953	UP
galT	2.5348148	2.5013621	UP
glbB	1.9762061	1.7950336	UP
glbD	1.5911142	1.5978236	UP
glbF	2.4462694	2.7040259	UP
glyA	2.4103821	2.5744876	UP
gntT	1.5534783	1.8202428	UP
groL	2.3772615	2.3177023	UP
guaA	1.7488026	1.8788231	UP
guaB	2.766507	2.6966152	UP
hisA	2.0279168	2.2686194	UP
hisB	2.1200549	1.9372098	UP
hisC	2.262883	2.0937	UP
hisD	2.1417726	1.906253	UP
hisF	1.8078847	2.0123219	UP
hisH	2.3688223	2.3198453	UP
hisI	1.7144812	1.8300672	UP

ileS	1.3032226	1.5116992	UP
ilvD	1.59771	1.4398928	UP
ilvI	1.4522486	1.786143	UP
ispH	1.3954088	1.1530124	UP
livJ	1.5470831	1.8789906	UP
lspA	1.0450108	1.2211087	UP
mepM	1.9508274	1.7224468	UP
mnmg	1.5303939	1.4188948	UP
mgo	1.9579611	2.5872277	UP
mrcA	1.2213919	1.3718811	UP
mukB	1.2426668	1.1104389	UP
ndk	2.2807822	2.1217788	UP
ompF	2.5626393	3.48055	UP
pal	1.217048	1.136431	UP
parC	1.7372547	1.682785	UP
pdeI	1.7289128	2.0429775	UP
plaP	1.8732439	2.2182744	UP
pncC	1.5179967	1.5083757	UP
prfC	1.2818753	1.410118	UP
priB	1.9634638	1.8864459	UP
proS	1.2039357	1.3091838	UP
prs	1.7898434	1.5614935	UP
purL	1.6033868	1.8217504	UP
pyrB	2.0198238	2.1597259	UP
pyrC	2.0650146	1.888299	UP
queA	1.6254811	2.0917424	UP
radA	1.9701095	2.2028755	UP
rlmL	1.3806352	1.1769768	UP
rnb	1.7685429	1.8247508	UP
rph	1.5396935	1.7403985	UP
rplB	1.395819	1.82838	UP
rplI	1.4976119	1.2929544	UP
rplP	1.4258026	1.6654565	UP
rplV	1.348405	1.5537005	UP
rpsC	1.3210334	1.717477	UP
rpsR	1.8586121	1.7548581	UP
rpsS	1.1814064	1.5895741	UP
rpsT	2.157139	2.2078592	UP
ryeA	3.0968058	3.4526017	UP
serB	1.919514	2.1895088	UP

speC	1.7314021	1.8405434	UP
sucB	1.4298788	1.979113	UP
suhB	2.3215631	2.7777886	UP
tgt	1.5044486	1.8169918	UP
tktA	1.7096646	1.8945979	UP
tufB	1.5478817	1.5375242	UP
tyrA	1.4871142	1.8578626	UP
yadS	1.3403986	1.2602719	UP
yafT	1.5777587	1.6508381	UP
yafX	1.478699	1.5711779	UP
yagF	1.3425526	2.9052264	UP
ybgD	1.3977031	2.1511433	UP
ydjO	2.3864902	1.9681787	UP
yegD	1.3421354	1.5734219	UP
yffS	3.6820256	3.2507594	UP
yhgF	1.6207437	1.6682573	UP
yifK	1.1337057	1.2696578	UP
yjgN	1.2450638	1.4181303	UP
yoaB	1.5161056	1.3189064	UP
yojI	1.8697513	1.7273446	UP
yqgC	1.9376969	1.8762253	UP

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