Supplementary material

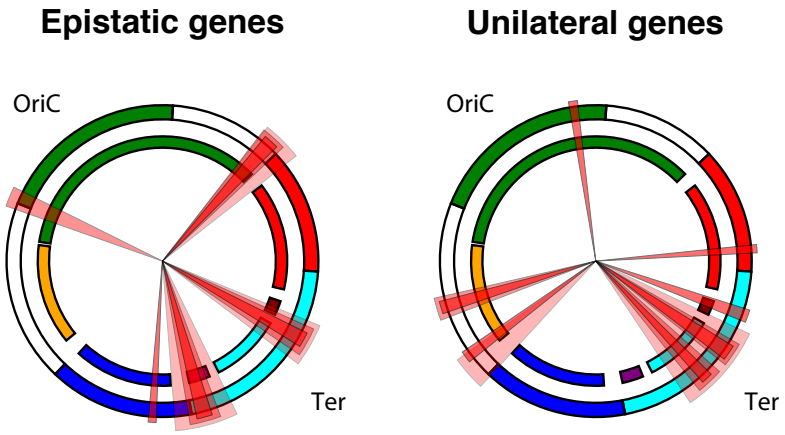
Supplementary Methods

Generation of *Δrac-stpA-hns* and *Δcps-hns-stpA* triple knockouts

Δrac-stpA-hns knockout was generated sequentially by deleting *stpA* in *E. coli* MG1655, then the *rac* prophage, followed by the deletion of *hns* in the *Δrac-stpA* double mutant. All these deletions were done by λ red recombinase method described by Datsenko and Wanner [1]. After every deletion, the kanamycin cassette inserted in place of the gene was cured by using pCP20 plasmid, and retransformed with pKD46 to promote further deletions.

The *Δcps-hns* double knockout was generated by deleting *hns* in a *E. coli* MG1655 *Δcps* background; both *Δcps* and the *Δcps-hns* double mutant were made by the λ red recombinase method. Finally, *stpA* was deleted in the *Δcps-hns* mutant using P1 phage transduction of *ΔstpA::kan*^{r} into *Δcps-hns* as described by Thomason et al [2].

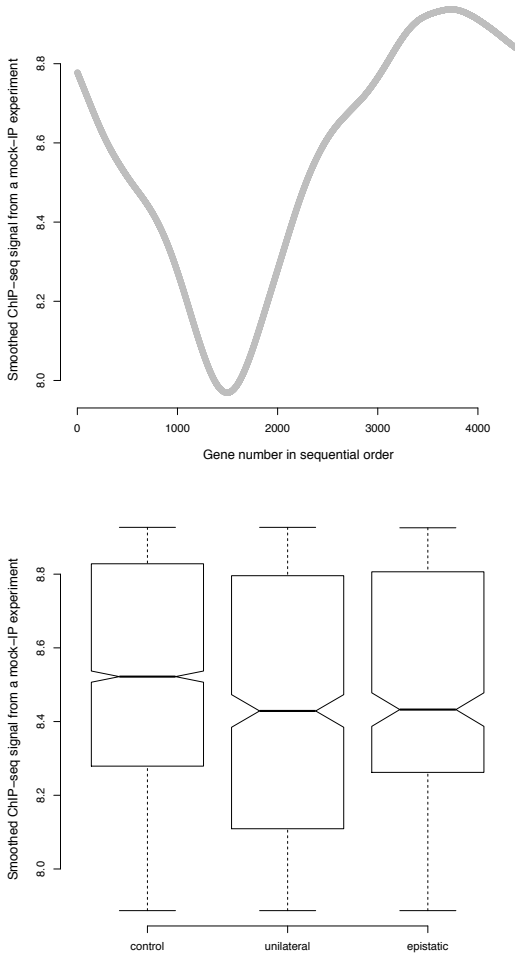
Supplementary Figure 1



The figure shows statistically significant clusters of epistatic and unilateral genes in relation to their chromosomal positions. These figures were drawn using the NuST web server [3]. The clusters are represented by the red coloured wedges, with the transparency of the colour increasing with the size of the cluster. The outer circle shows the macrodomains described by Valens and colleagues based on the observation that loci within a macrodomain are more likely to recombine with each other than across macrodomains [4] (green: ORI macrodomain; red: RIGHT macrodomain; cyan: TER macrodomain; blue: LEFT macrodomain; white: non-structured regions). The inner circle shows the various chromosomal sectors, computationally defined as regions showing a positive correlation between transcriptional level and a measure of codon adaptation index [5].

Epistatic genes are clustered at certain macrodomain boundaries, primarily those between the TER and the LEFT macrodomains, and between the RIGHT and its proximal non-structured region. Unilateral genes are spread through the body of the TER macrodomain.

Supplementary Figure 2



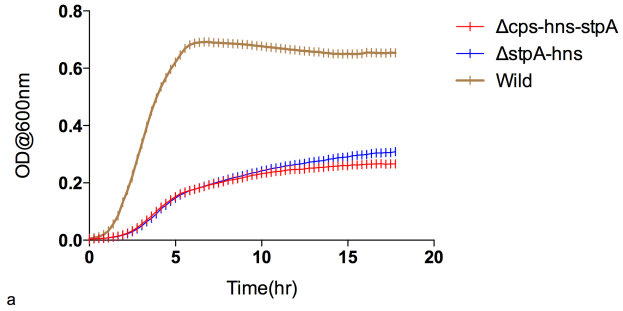
The top panel shows the ChIP-seq signal (y-axis) from a mock-IP experiment published previously [6], as a function of gene position (x-axis; the number represents the ORF number, ordered sequentially, with the terminus located at ˜ORF #1,500 and the origin around ORF #3,700). The signal across the gene body was calculated as described in the paper describing the original experiment, with smoothing performed using the smooth.spline function in R. The bottom panel shows box plots of the distribution of the above mock-IP signal for epistatic, unilateral and control genes.

Supplementary Figure 3

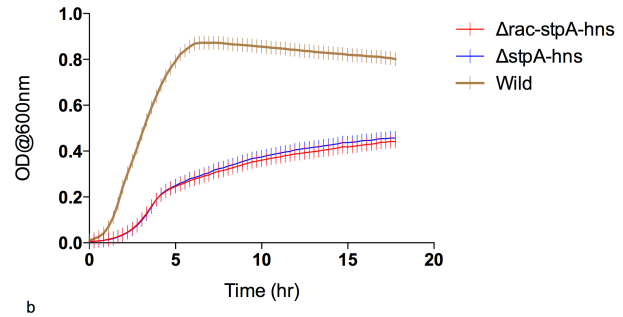
A previous study, with the objective of investigating the role of H-NS and StpA in virulence gene expression in pathogenic *E. coli*, again showed a growth defect in the double mutant [7]. These authors reported that the distinctive feature of the *ΔstpA-hns* transcriptome was the down-regulation of metabolic genes, allowing them to propose an association between this and the growth phenotype. In the present study, we notice that ~50 genes are downregulated in *ΔstpA-hns* when compared to both *Δhns* and the wildtype. The down-regulation of expression of any gene that is essential for *E. coli* growth in LB medium could in principle cause a reduction in growth rates. However, the ~50 down-regulated genes included only one of ~300 essential genes described by Baba and colleagues [8]. This gene is *racR* - a putative transcription factor encoded within a cryptic Rac prophage - whose deletion is lethal to *E. coli*, on the basis of several failed attempts - including ours - to obtain this mutant [8]; this gene is down-regulated by as much as 10-fold in the double mutant when compared to the wildtype. Though *ΔracR* itself is lethal, deletion of the entire Rac prophage is not [9], suggesting that an unknown factor responsible for the lethality is encoded in the prophage. If this factor were responsible, at least in part, for the growth defect of *ΔstpA-hns,* then a triple knockout of *ΔstpA-hns* in a background strain lacking the entire Rac prophage should at least partially restore wildtype growth. However, a triple deletion - *Δrac-stpA-hns* - we obtained showed growth similar to that of the *ΔstpA-hns* double mutant (Supplementary Figure 1, Supplementary Methods). A possible explanation for the apparent lack of an effect of *racR* down-regulation on growth is that the putative lethal factor within the prophage may also be down-regulated in *ΔstpA-hns*, thus compensating for the reduced availability of RacR.

We also tested whether one striking case of transcriptional up-regulation might be responsible for the growth defect of the double mutant. This follows from the study by the Hinton group [10] in which it was demonstrated that a growth defect in a *Δhns* strain in *Salmonella* could be partially reversed in a strain background lacking a type III secretion system, a horizontally-acquired region that is silenced by H-NS. In our double mutant transcriptome data, we noticed strong up-regulation of several genes involved in the biosynthesis of polysaccharide antigens, which might divert nutrients from energy metabolism towards biosynthesis. Many of these genes are clustered in a single locus [11]. This ~35kb cluster can be divided into two. The first ~10kb is involved in lipopolysaccharide O-antigen biosynthesis, which is silent because of the presence of an IS5 insertion (called rfb-50 mutation). The second ~23kb (called *cps* locus here) encodes genes for the synthesis of capsular polysaccharide colanic acid. Whereas the transcriptional up-regulation seen for the O-antigen region is ~2-4-fold, that for genes in the colanic acid region is generally over ~10 fold. Several binding sites for H-NS could be detected in this region from previously published ChIP-seq data. ChIP-qPCR experiments performed by us further showed binding of H-NS and StpA to these loci, irrespective of the strain background (wildtype / *ΔstpA* / *Δhns*; data not

shown). We were able to generate a *Δcps* strain, on top of which we made the *ΔstpA-hns* double mutant. This however failed to reverse the *ΔstpA-hns* growth defect suggesting that any contribution this might make to fitness is not easily perceptible (Supplementary Figure 1, Supplementary Methods). However, this is not to rule out the possibility that uncontrolled up-regulation of toxic genes might contribute to the double mutant's growth phenotype.



a



b

The above graph shows the growth curve of triple mutants *Δcps-stpA-hns* (top) and *Δrac-stpA-hns* (bottom) in comparison with *ΔstpA-hns* and wildtype *E. coli* MG1655. No rescue of the *ΔstpA-hns* growth phenotype was observed on deletion of the *rac* prophage or the capsular polysaccharide (cps) genes in *ΔstpA-hns* background.

References

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