Inhibition of factor-dependent transcription termination in *Escherichia coli* might relieve xenogene silencing by abrogating H-NS-DNA interactions *in vivo*

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Supplementary material

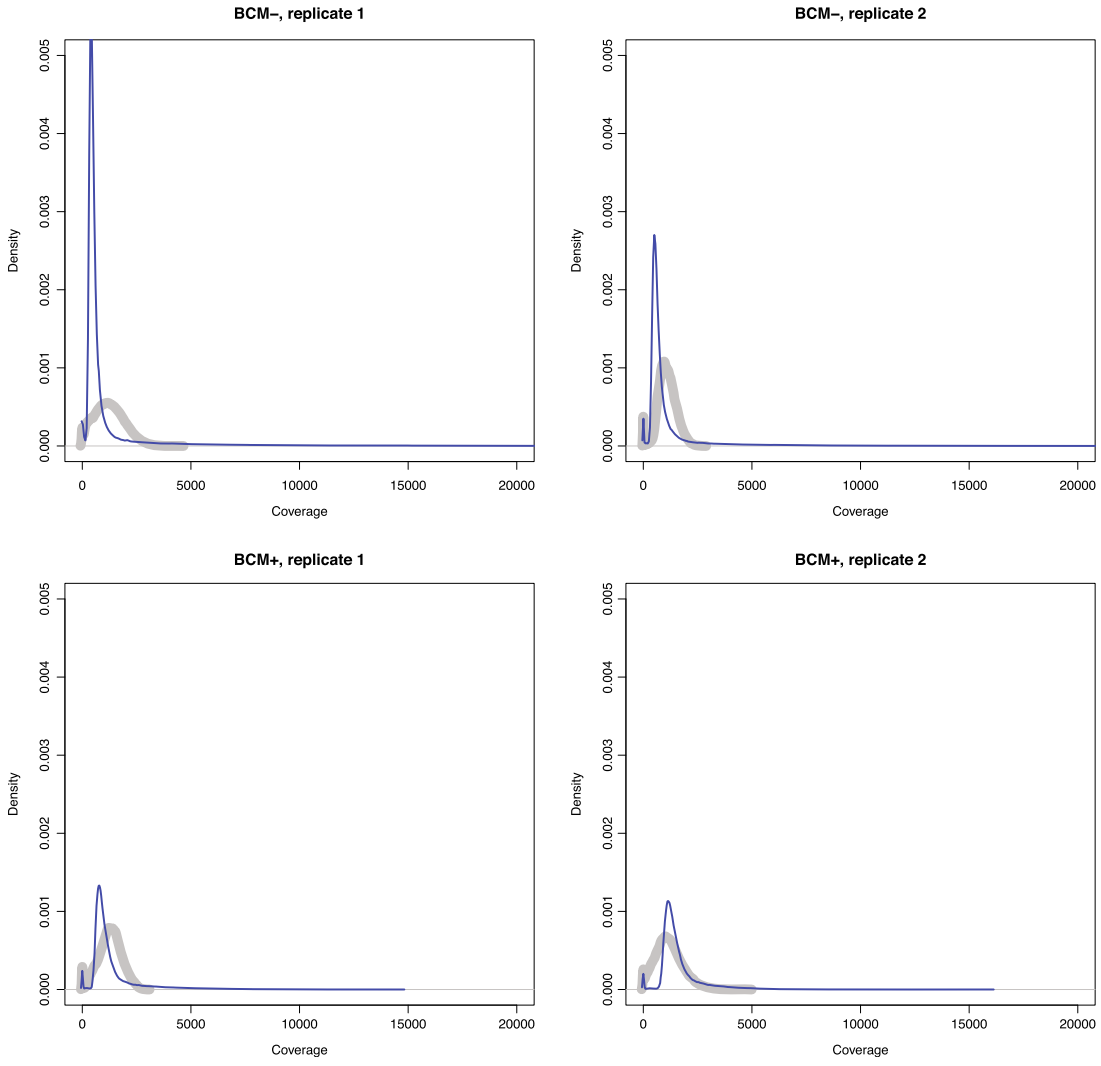
Chromatin immunoprecipitation

MG1655 *hns*::3xFLAG cells were grown in liquid LB medium with and without bicyclomycin (BCM). For samples without BCM treatment, cells were grown to $OD_{600} \approx 0.8$ (≈4 h) at 37°C with shaking at 200 rpm. For samples with BCM treatment, cells were grown to early log phase (O.D. 600=0.1) ≈ 1h at 37°C with shaking at 200 rpm. BCM was added to a final concentration of 25 μg mL^{-1} and cells were grown for an additional 3h. Formaldehyde (1% final concentration) was added to the culture flask and incubated for 20 min at 37°C, 200 rpm. The reaction was quenched by adding glycine (0.5 M final concentration) and incubated under same conditions for 5 min. 20 mL of cross-linked cells were pelleted by centrifugation for 10 min at 2500*g* and washed twice with ice-cold TBS (pH 7.5). The pellet was suspended in 1 mL TBS and transferred into an 1.5 mL eppendorf tube and spun for 2 min. The supernatant was removed and cells were snap-frozen in liquid nitrogen. The pellet was stored at −80°C to be used for subsequent steps. The stored pellet was resuspended in 1 mL of lysis buffer [10 mM Tris (pH 8.0), 20% sucrose, 50 mM NaCl, 10 mM EDTA, 20 mg/mL lysozyme and 0.1 mg/mL RNase A] and incubated at 37°C for 30 min. The components of lysis buffer help break open the bacterial cell wall. After the lysis, 3 mL immunoprecipitation (IP) buffer [50 mM HEPES–KOH (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS) and PMSF (final concentration 1 mM)] was added and two microlitres of lysate was sheared using bioruptor to a size of 200 – 300 bp with 40 cycles of 30 s ON/OFF at high setting. Water (ice-cold) was changed every 5 cycles. Insoluble cellular matter was removed by centrifugation at 10 min at 4°C. The supernatant collected was subjected to an additional centrifugation step with the same conditions mentioned to ensure complete removal of insoluble matter, which otherwise might block the column in the subsequent steps. Further the supernatant was divided into two 800 μl aliquots. The remaining 400 μl was kept to check the size of the DNA fragments also further purified to be used as input DNA.

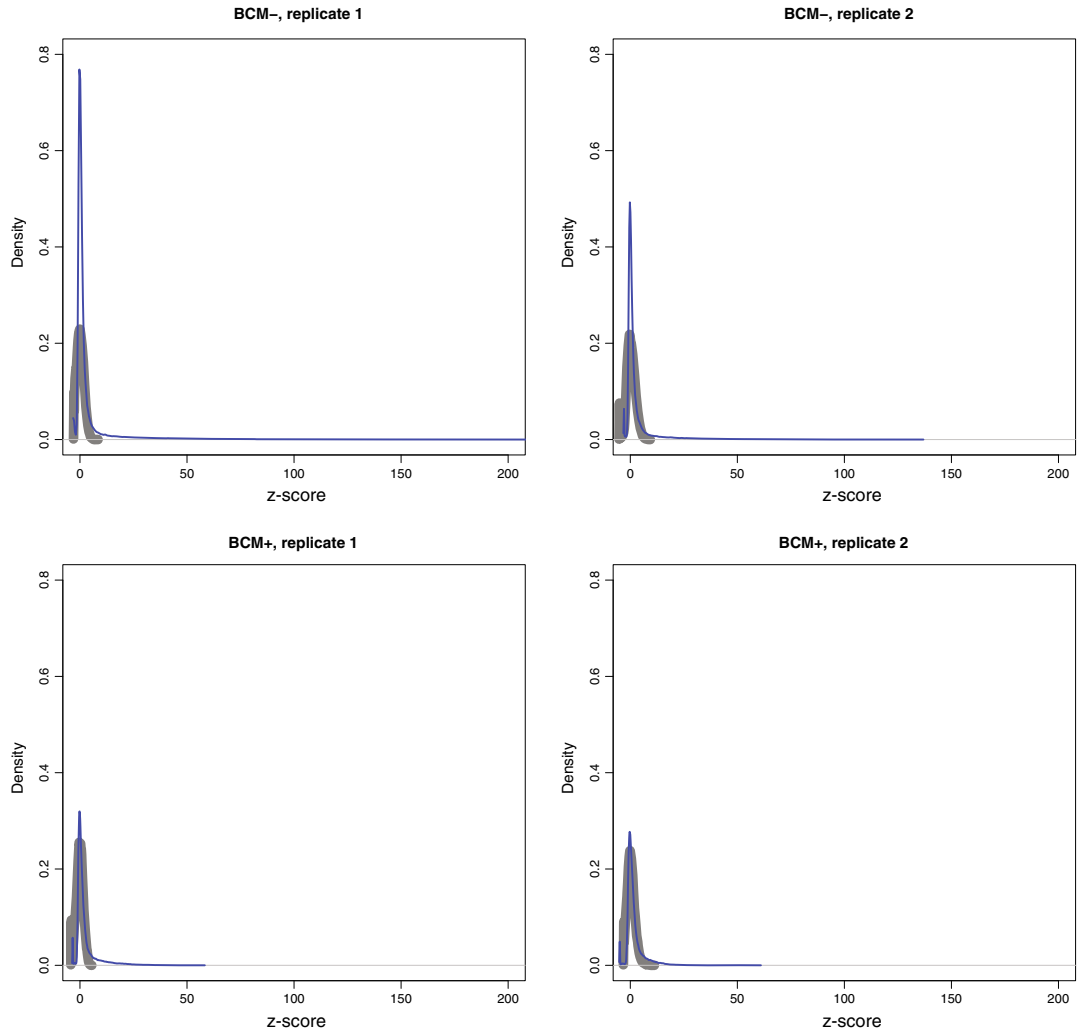
Each 800 μl aliquot was incubated with 20 μl Protein A/G sepharose beads (Pierce Cat # : 53132) for 45 min at room temperature on rotary shaker to get rid of non specific complexes binding to the resin. The supernatant was removed and transferred into fresh 1.5 mL tube. Following which the tubes with supernatant was incubated with monoclonal anti-FLAG antibody (Sigma Cat #: F3165-1MG) and no antibody (mock-IP, where required) on rotary shaker, room temperature for 1 hr. During incubation, 30 μl Protein A/G UltraLink Resin was washed and blocked with 1mg/mL bovine serum albumin (BSA) in TBS. The blocked beads, protein-DNA complexes and antibody were incubated on a rotary shaker at 4°C overnight.

The sepharose beads were collected using 0.22 μm Spin-X centrifuge tubes (Costar Cat#: 8169). Samples were washed once with IP buffer and twice with high salt IP buffer (IP buffer + 500 mM NaCl), once with wash buffer [10 mM Tris (pH 8.0), 250 mM LiCl, 1 mM EDTA, 0.5% Nonidet P-40 and 0.5% sodium deoxycholate] and with TE-buffer (pH 7.5). Immunoprecipitated complexes were eluted in 100 mL elution buffer [10 mM Tris (pH 7.5), 10 mM EDTA and 1% SDS] at 65°C for 20 min. Additional elution step was carried out with 25 μl of elution buffer.

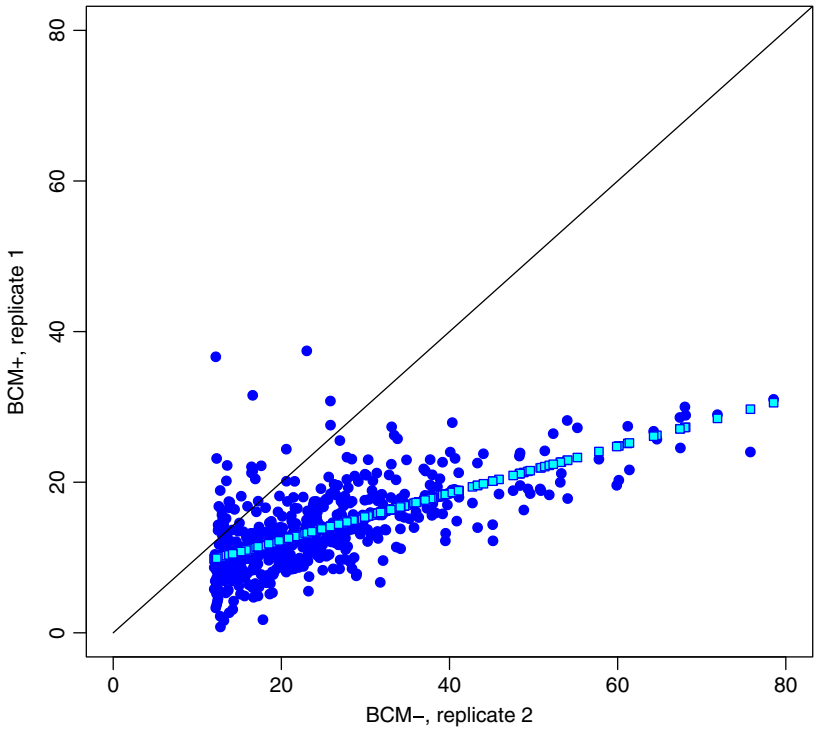
Immunoprecipitated samples and the sheared input DNA were de-cross linked in 0.5X elution buffer containing 0.8 mg/mL Pronase at 42°C for 2 h followed by 65°C for 6 h. DNA was purified using a PCR purification kit (QIAGEN).



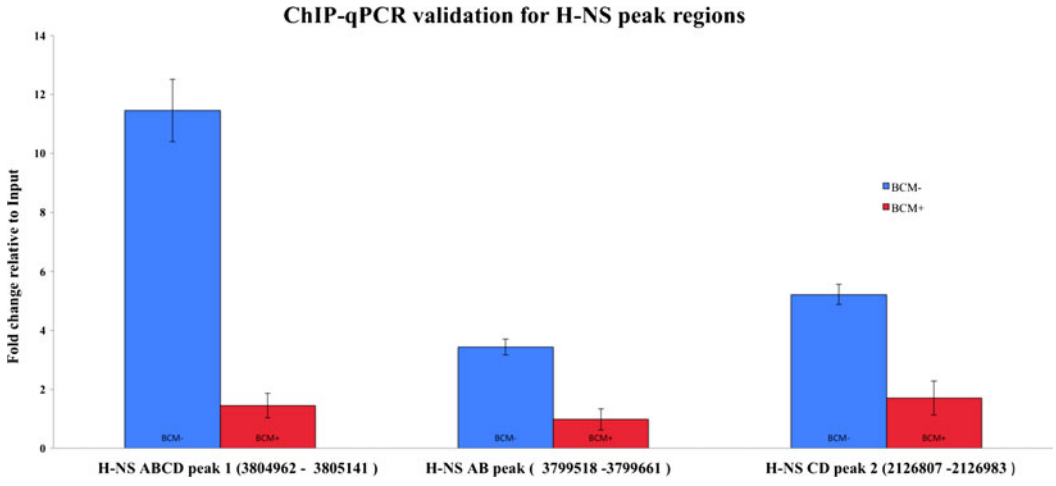
Supplementary figure 1. This figure shows the distribution of read coverage for the two BCM+ and BCM– replicates. The blue line shows the ChIP data, whereas the thick grey lines represent the matched input DNA samples. The position of the crest of each of these distributions depends on the read coverage for the respective sample.



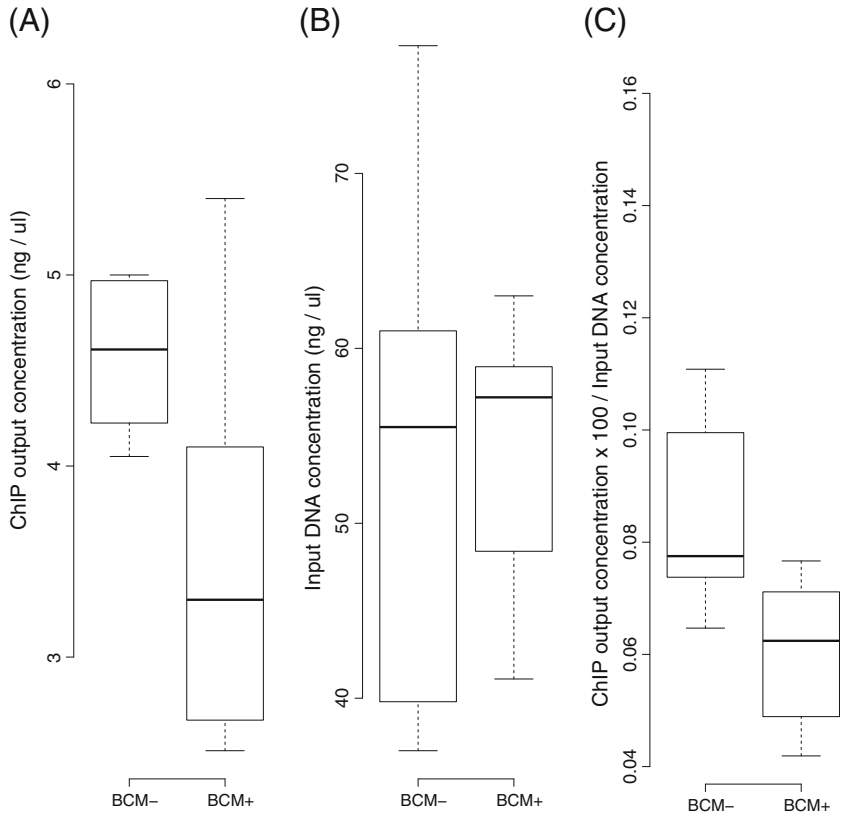
Supplementary figure 2. This figure shows the distribution of z-scores for the two BCM+ and BCM– replicates. The blue line shows the ChIP data, whereas the thick grey lines represent the matched input DNA samples.



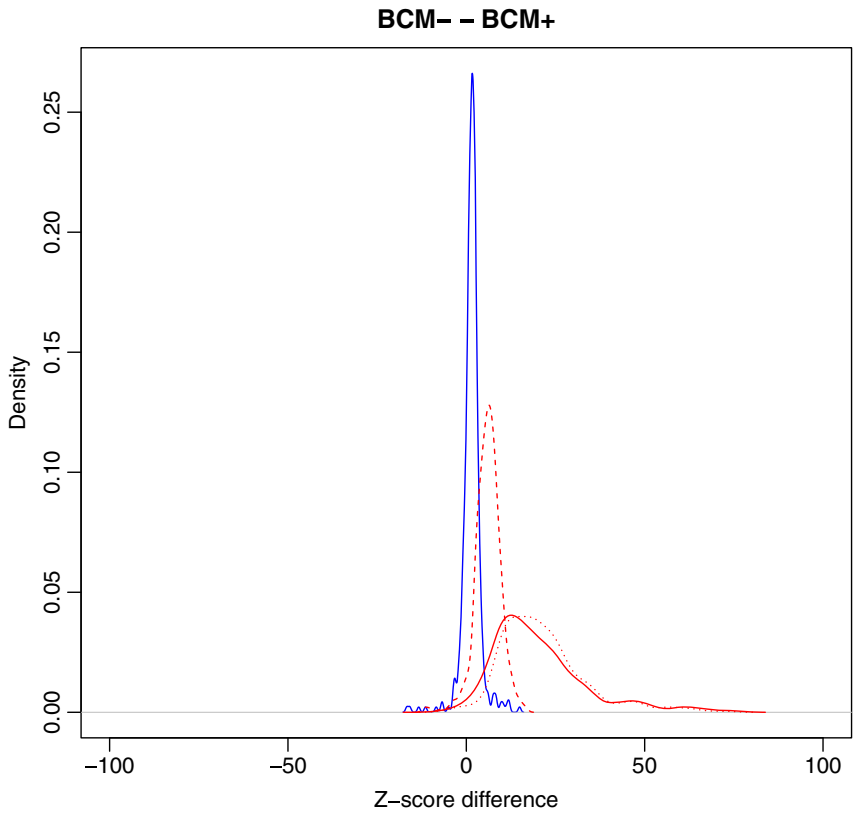
Supplementary figure 3. This figure shows a scatter plot (filled blue circles) of the binding signal between a BCM− and a BCM+ replicate. The cyan rectangles follow the best-fit line. The replicate showing the weaker signal for BCM and that showing the stronger signal for BCM+ were chosen for this plot. Of the four possible comparisons between BCM+ and BCM− (2 x replicates each for BCM+ and BCM−), this comparison shows the least difference between BCM− and BCM+. The 45°C line is also drawn in black.



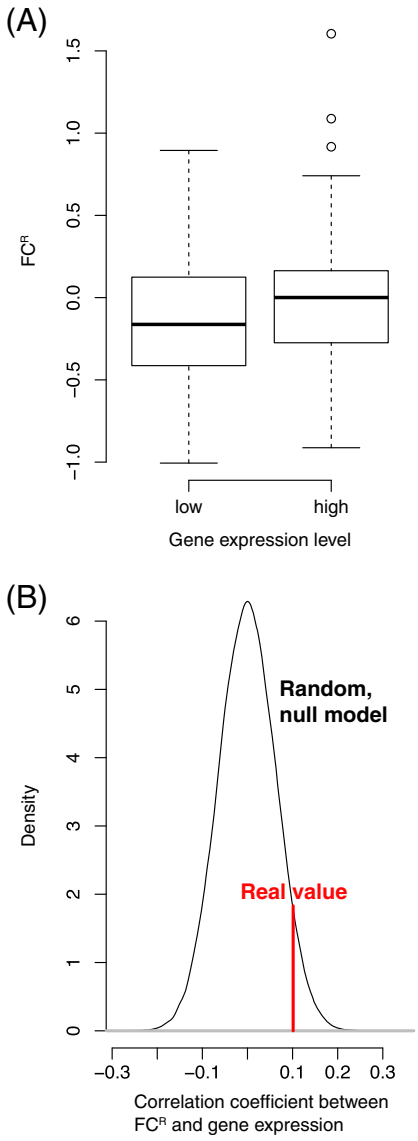
Supplementary figure 4. This figure shows the fold change between the ChIP product and the input for three distinct H-NS binding regions for BCM− (blue) and BCM+ (red). The genomic coordinates of the PCR amplicons are marked along the x axis. These regions are as described previously by Kahramanoglou et al. The error bars represent the range across four ChIP experiments. Four technical replicate ChIPs, across two independent cultures for each treatment (in addition to those performed for the sequencing experiment) were used to test for difference in signal between BCM− and BCM+ samples with the help of qPCR against three H-NS binding regions. These were performed on the same cell population used for the ChIP-seq experiments. These support the conclusions of the ChIP-seq analysis.



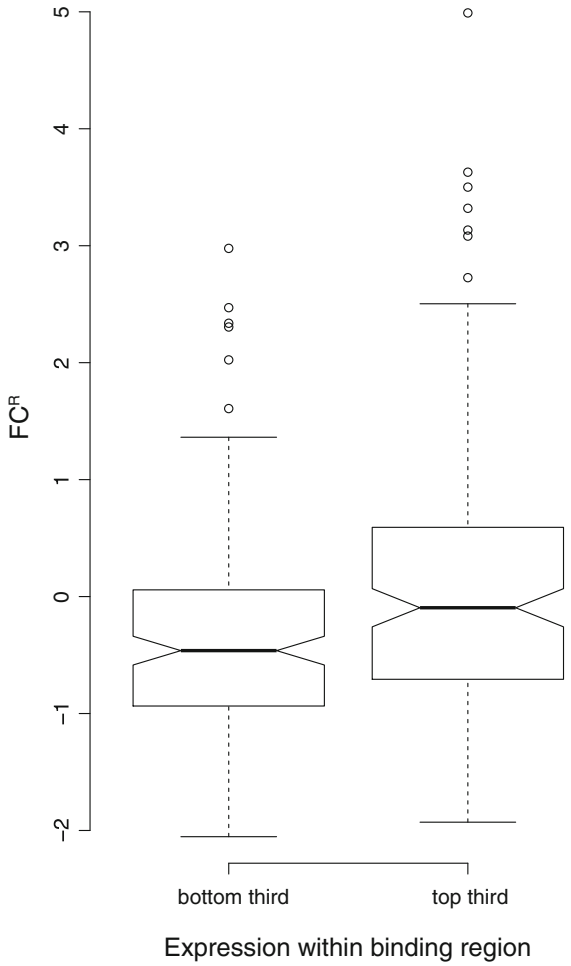
Supplementary figure 5. This figure shows the concentration of DNA for (**a**) ChIP experiments and (**b**) input for BCM− and BCM+ cultures. Panel (**c**) shows the ratio of the ChIP DNA concentration and the corresponding input DNA concentration. The data are over eight replicates. There is little difference between BCM+ and BCM− in terms of the concentration of input DNA, but BCM+ cultures generally yield less ChIP product than BCM−.



Supplementary figure 6. This figure shows the distributions of differences in Z-scores between two ChIP / mock-IP experiments. These were calculated over the H-NS binding regions identified in both BCM– replicates. The solid blue line compares one biological replicate mock-IP experiment between BCM− and BCM+. The solid red line is for H-NS ChIPs comparing BCM− and BCM+. The red dotted line represents the difference between the ChIP and the mock-IP for BCM− and the red dashed line for BCM+.



Supplementary figure 7. (**a**) Shows the distributions of FC^R for binding regions surrounded by loci of low gene expression (bottom 25%; left), and those by loci of high gene expression (top 25%; right). FC^R is defined as the residual of the linear regression fit between $\log_2$(BCM+) – $\log_2$(BCM+) and $\log_2$(BCM−), where BCM+ and BCM− refer to the ChIP-seq determined binding strengths of H-NS in BCM+ and BCM− cells respectively. (b) Shows the distribution of correlation coefficients between randomized (N = 100,000) FC^R and the expression level of loci surrounding HNS binding regions. The randomization was done by shuffling the lists of FC^R and expression levels, such that any association between the two is lost. The red vertical line marks the correlation coefficient between the real FC^R and expression levels. >94% of all correlation coefficients in the randomized list are below this level. The expression data are from RNA-seq experiments reported by us in Srinivasan *et al.* (2013).



Supplementary figure 8. Shows the distributions of FC^R for binding regions showing low gene expression (bottom 33%; left), and those with loci of high expression (top 33%; right) in the Δhns-stpA knockout strain. The expression data are from RNA-seq experiments reported by us in Srinivasan *et al.* (2013).