Supplemental Methods

Growth assay. Aerated bacterial cultures were grown from frozen stocks at 37°C overnight. A 1:100 dilution of this overnight culture was used for growth curve experiments by measuring optical density (OD) at 600 nm at specified time points. To determine the CFU at the final time point, 1 ml of culture at OD_{600} = 2.00 was centrifuged, resuspended in 1ml 1X sterile phosphate-buffered saline (PBS) and serial dilutions were plated, with appropriate antibiotic selection where needed. Growth curves and CFU were plotted using Prism software (GraphPad, v. 5.01). Result represents two independent experiments.

Detailed method for *P. mirabilis* ChIP and PCR-walk. This ChIP protocol was developed for *P. mirabilis* and has been guided by published protocols (1-3). Immunoprecipitation of *P. mirabilis* nucleoprotein was started with the seeding of 50 ml of LB supplemented with ampicillin with a 1:100 dilution of an overnight culture and was grown at 37°C with 225 rpm agitation. Cultures were induced by the addition of 0.002% arabinose at OD_{600} = 0.5. Cells were harvested in stationary phase (OD_{600} = 2) by centrifugation at 1500 X *g* for 15 min. Bacterial pellets were snap frozen at -80°C; separate aliquots were kept and tested by western blot for MrpJ as a control before carrying out each ChIP assay.

Pellets were thawed on ice and washed twice in 5 ml sterile 1X PBS (pH 7.4) prior to resuspension in 10 ml final volume. 37% formaldehyde solution (Fisher Scientific) was added as an *in vivo* cross-linker to reach a final concentration of 1% and the tubes were gently rocked for 10 min at room temperature followed by the addition of glycine (final concentration of 0.75M) to cease cross linking (5 min rocking at room temperature). Cross-linked cells were washed twice in 5 ml sterile 1X PBS at 4°C. Pellets were resuspended in 10 ml ice-cold sterile lysis buffer (1% Triton X-100, 1 mM EDTA, 15 mM Tris-Cl pH 7.5, 150 mM NaCl) fortified with protease and phosphatase inhibitors (Roche Complete Mini, PMSF). A French press was used to mechanically lyse the cells using two passes at 1500 psi and the resulting lysate was centrifuged twice at 16,000 X *g*, 10 min at 4°C to get rid of cellular debris. Cleaned lysates were distributed in 500 µl aliquots and sonicated (Diagenode Bioruptor) at 4°C for a total of 20 cycles (with 30 sec ON and 30 sec OFF time; final fragment lengths were enriched in the range of 200-650 bp). Samples were quickly vortexed and centrifuged, and the supernatants were transferred into new pre-chilled tubes after the end of the first 10 cycles. The sonicated lysates were further cleared by centrifugation twice (16,000 X *g*, 10 min,4°C). 400 ul of clean lysate was added onto 30 ul of previously prepared 50% UltraLink Protein A/G agarose bead slurry (Pierce/Thermo Fisher) to be pre-cleared for 2 hours with gentle rotation at 4°C. Beads were prepared earlier by washing twice in 1X sterile PBS (pH 7.4), equilibration in ChIP lysis buffer for 10 min with gentle rotation, followed by blocking for 1 hour in 0.1% BSA in ChIP lysis buffer and washing three times in ChIP lysis buffer after blocking; beads were collected at each step by centrifugation (2 min, 500 X *g*, 4°C). The beads were gently spun down after pre-clearing and the clean lysate supernatants were aspirated and pooled together before being distributed for immunoprecipitation (IP); 100 µl of this lysate was saved at -80°C as pre-IP input (In).

40 µl of prepared beads (washed, equilibrated and blocked) was conjugated overnight with 1.5 µg of anti-His antibody in 320 µl of ChIP lysis buffer (gentle rotation at 4°C) one day in advance. These antibody-conjugated beads were washed three times in lysis buffer and 320 µl of pre-cleared lysate was added per IP reaction followed by an overnight incubation at 4°C with gentle rotation; a no antibody bead-only control was included for each lysate type used in this study. In parallel to this, an 'antibody coupled with lysate' IP reaction was carried out overnight (320 µl lysate plus 1.5 µg of anti-His antibody) for each lysate type described. The next morning, these antibody-lysate IP reactions were added onto 40 µl of previously prepared beads and gently rotated for 5-6 hours at 4°C. Next, all IP reactions were washed in 800 µl of pre-chilled wash buffers (specified below) to get rid of non-specific background and to enrich for specific

MrpJ-DNA interactions. The order and the number of washes were: 3X in ChIP lysis buffer, 1X in high-salt buffer (500 mM NaCl in lysis buffer), 1X in low-salt buffer (250 mM LiCl in lysis buffer), 2X in TE (10 mM Tris, 1 mM EDTA); tubes were changed before the last lysis buffer wash as well as before the last TE wash. All wash steps were carried out for 5 min at 4°C with gentle rotation and the beads were collected by 2 min centrifugation at 500 X *g*. At the end, one last spin was done to collect any residual TE and the supernatant was gently aspirated to collect only the immunoprecipitated-bead pellet for the elution step. Next, the DNA-protein complexes were eluted off of the beads in 200 µl of freshly prepared elution buffer (50 mM $NaHCO_3$, 1% SDS in sterile dH_2O) by gentle vortexing for 15 min (at vortex speed 3) at room temperature. Eluted nucleoprotein complexes were collected by centrifugation (2000 rpm, 5 min, room temperature), 192 mM NaCl was added, and cross-linking was reversed at 65°C overnight in a hybridization oven; previously saved pre-IP input samples (10% of IP, 32 µl) were also included for cross-linking reversal.

The next day, all samples were purified using Enzymax DNA spin columns. PCR reactions were performed to evaluate MrpJ-DNA interactions on *mrpA* and *flhD* upstream regulatory elements including their respective promoters to probe for *in vivo* binding. All primers used for this chromosome-walk are listed in Table S2. Cycle conditions: 94°C 1m, [94°C 15s, 52°C 30s, 72°C 30s] x 30, 72°C 2m, 4°C ∞; with an exception of 1m 30s elongation and amplification for 35 cycles with *mrp* amplicons. PCR products were resolved by 2% agarose gel, stained in ethidium bromide solution and visualized by a gel documentation system. Semi-quantitative estimation of band intensities was performed using Image Studio Lite software (LI-COR). Three independent ChIP experiments were performed.

References (including Supplemental Tables)

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