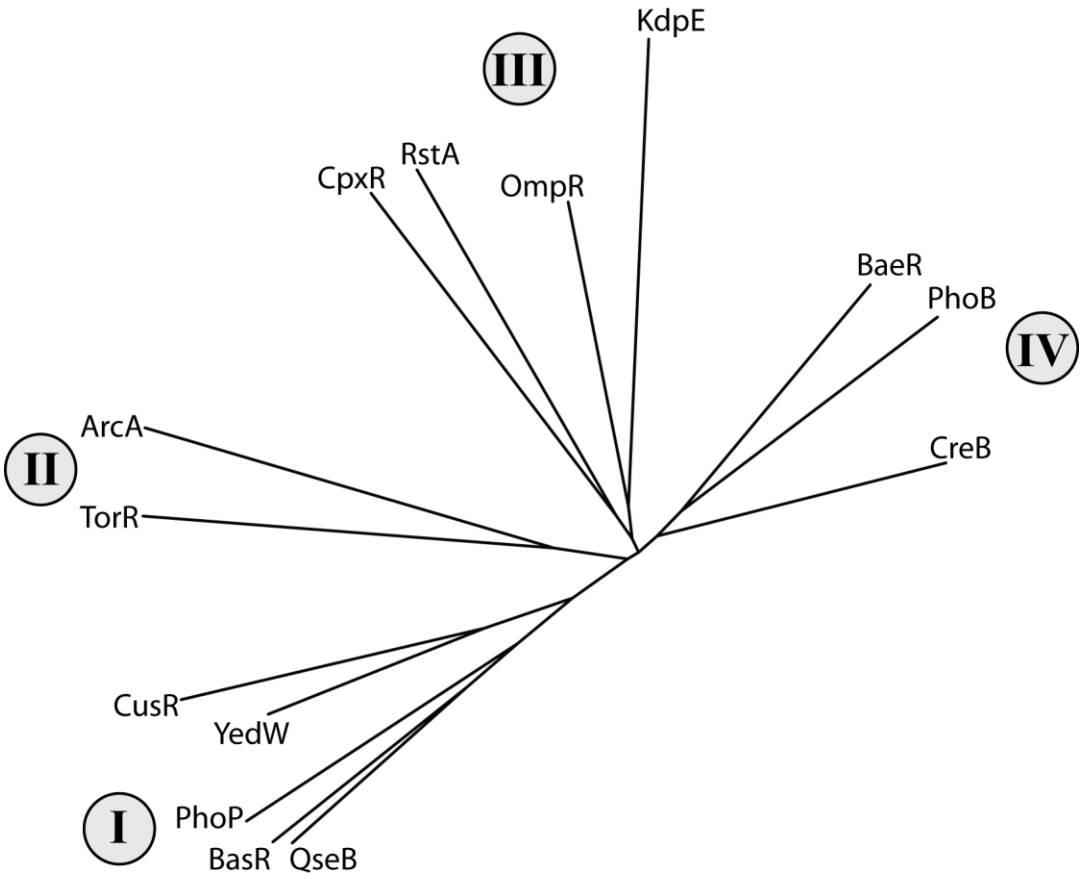

Figure S1. Phylogenetic tree of OmpR family represented in *E. coli*. A phylogenetic tree was constructed based on consensus representations of eRRs. Lineages I–IV correspond to Figure 1C.

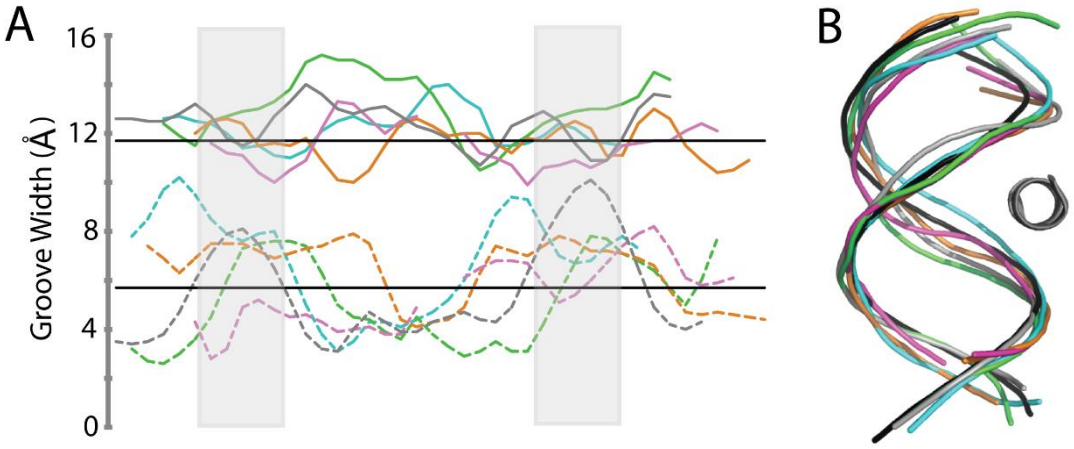

Figure S2. DNA shape varies between OmpR family RRs. (A) Groove widths for DNA major (solid lines) and minor (dashed lines) are displayed for DNA sequences in complex with dimeric RR: RstA (PDB code: 4NHJ [1], magenta), KdpE (PDB code: 4KNY [2], green), PhoB (PDB code: 1GXP [3], cyan), PhoP (PDB code: 5ED4 [4], orange), PmrA (PDB code: 4S05 [5], gray). DNA half-site sequences were aligned based on a structural overlay of bound RR monomers (a gap was introduced in the spacer region for RstA to account for its reduced half-site spacing). Horizontal black lines display the average B-form width parameters for the major (11.7Å) and minor (5.7Å) grooves. Gray boxes

indicate the central 3 bases of each half-site. (B) Recognition helices from a representative RR monomer from each structure were aligned in PyMol [6] to normalize the relative angle of groove entry, and the resultant phosphate backbone trajectories are represented according to the color scheme described in Panel A.

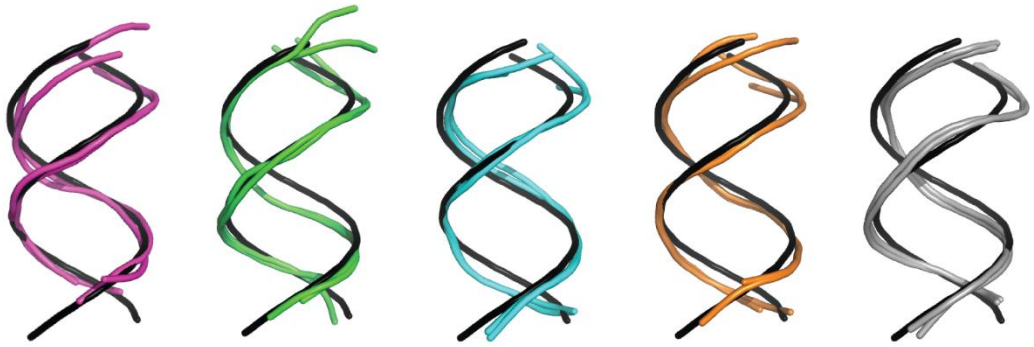

Figure S3. Deviation of phosphate backbone induced by RR binding. Each structure presents an overlay of bound half-sites (colored) and a DNA structure corresponding to ideal B-form parameters (black). The RH of upstream and downstream monomers in five available RR:DNA structures were aligned (as in Figure S2B) to display the relative positioning of DNA structures. Structures and coloration correspond to Figure S2B: RstA (PDB code: 4NHJ [1], magenta), KdpE (PDB code: 4KNY [2], green), PhoB (PDB code: 1GXP [3], cyan), PhoP (PDB code: 5ED4 [4], orange), PmrA (PDB code: 4S05 [5], gray).

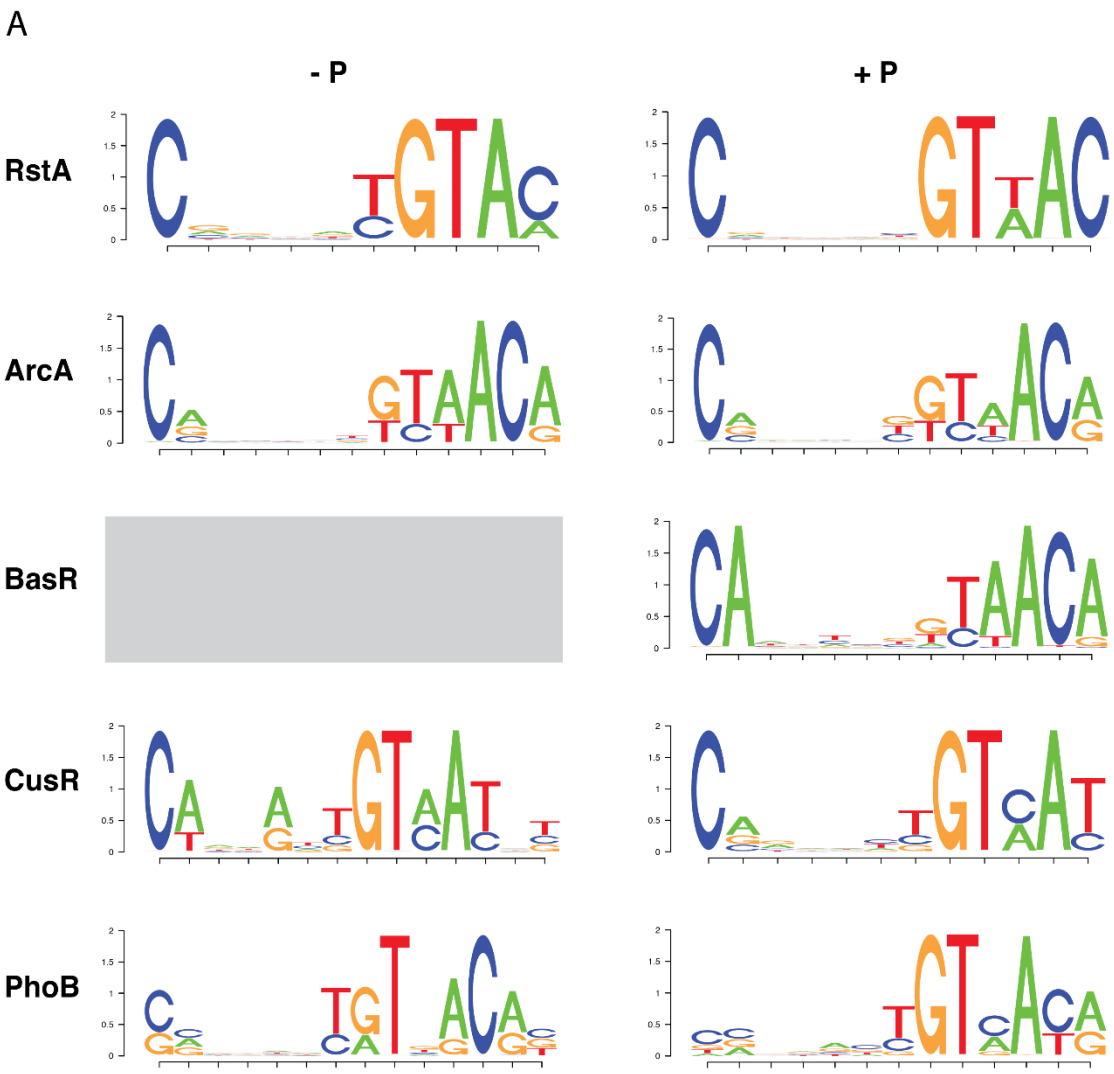

Figure S4. Binding orientation revealed by base preferences adjacent to fixed half-site sequence. Selected logos are the raw results of the de novo motif finding tool BioProspector [6], oriented to position the synthetic anchor sequence ('AGGTAA') to the left of each sequence logo.

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