



Figure S6: Synergistic effects of mutations combined with *lptB*-EHis₈ identifies a network of functionally-related residues. (A) Sequence logo generated, as described in Fig. 1D and Methods, of the conservation of residues in the signature, D-loop and switch helices (locations of each marked with thick lines). Below the logo, the sequence of the *E. coli* *LptB*^{WT} is shown in black and a summary of the functional analysis of single amino-acid substitutions to disrupt observed interactions between R144 and the CTD. Substitutions in pET23/42-*LptB* conferring no defects in strains carrying a chromosomal Δ *lptB* deletion are shown in green, those conferring partial loss of function are shown in orange, and those resulting in a total loss of function are shown in purple. (B) Ability of mutations that alter the indicated residue either in pET23/42*LptB* or pET23/42*LptB*-EHis₈ to complement a Δ *lptB* allele in the indicated media as described in Fig. S1A. (C) OM permeability of haploid strains carrying *lptB* mutations that exhibit synergistic effects with *lptB*-EHis₈. Disc diffusion assays were performed on LB plates. Numbers indicate diameter (in mm) of the zone of total growth inhibition or that of partial growth (in parenthesis). (D) LptB immunoblot of haploid *lptB*-EHis₈ strains carrying pET23/42-*LptB*-EHis₈ grown in LB. (E) LptB immunoblot of samples from haploid *lptB* strains carrying pET23/42-*LptB* grown in minimal medium to allow growth of strains carrying conditionally lethal alleles. WT refers to NR2101, which produces *LptB*^{WT} from pET23/42-*LptB*. (F) LptB immunoblot of samples from merodiploid strains carrying the wild-type *lptB* allele and mutant *lptB* alleles encoded in pET23/42-*LptB* grown in LB. 754 refers to haploid strain NR754, the wild-type strain with chromosomal *lptB*⁺; WT to NR2583, which produces *LptB*^{WT} from both the chromosome and pET23/42-*LptB*. (G) LptB immunoblot of samples from merodiploid strains carrying the chromosomal wild-type *lptB* allele and mutant *lptB*-EHis₈ alleles encoded in pET23/42-*LptB*-EHis₈ grown in LB. 754 refers to haploid strain NR754; WT refers to NR1872, which produces *LptB*^{WT} from the chromosome and *LptB*-EHis₈ from pET23/42-*LptB*-EHis₈. Data are representative of at least three independent experiments.