Farrand *et al.* Supplemental Material for Online Posting

Farrand *et al.* Figure S1

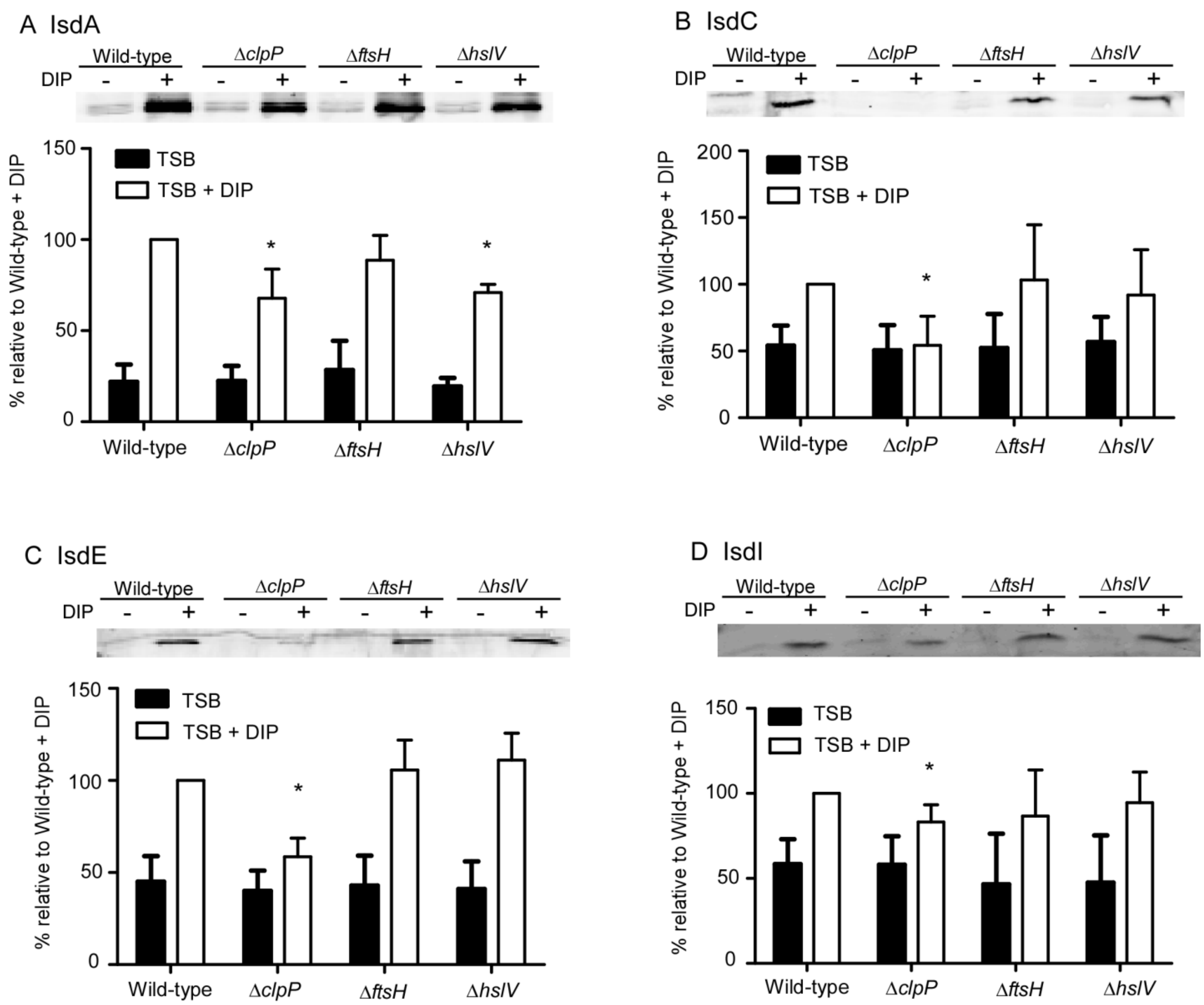

Figure S1. Disruption of the *S. aureus clpP* proteolytic subunit reduces abundance of additional Isd proteins. IsdA (**A**), IsdC (**B**), IsdE (**C**) and IsdI (**D**) protein levels analyzed by immunoblot. Wild-type *S. aureus* or strains inactivated for *clpP*, *ftsH,* or *hslV* were grown in iron rich (TSB) or iron poor (TSB + DIP) media. Total protein in cell wall (IsdA) or protoplast (IsdC, IsdE and IsdI) fractions were normalized and separated by 15% SDS-PAGE. Proteins were transferred to nitrocellulose and probed with antibodies directed to IsdA, IsdC, IsdE or IsdI. Blots are representative of 3 independent experiments (*above*). Graphical representation of Isd protein abundance assessed by densitometry in TSB (black bars) or TSB + DIP (white bars) from immunoblot analysis (*below*). Error bars represent SEM. Asterisk denotes $p < 0.006$ relative to wild-type in the respective condition as calculated by Student's *t*-test.

Farrand *et al.* Figure S2

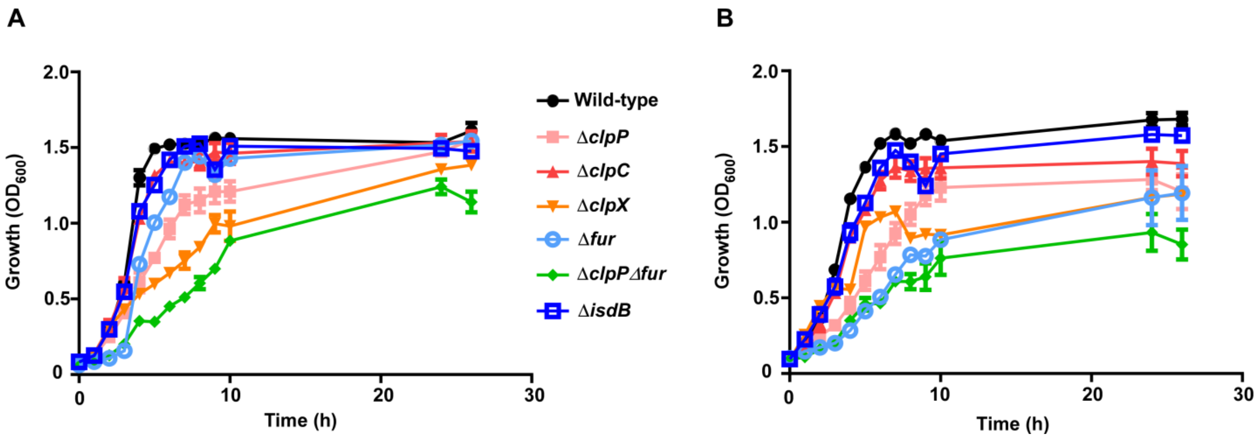

Figure S2. Growth of *clp* mutants in iron-replete and iron-deplete media. Wild-type *S. aureus* or strains inactivated for *clp, fur,* or *isdB* genes were grown in TSB (A) or TSB supplemented with the iron chelator EDDHA (1.5mM) (B). Bacterial growth was assessed every hour for 10 hours and at 24 hours by measuring the optical density of the cultures at 600 nm. Error bars represent SEM of triplicate samples.

Farrand *et al.* Figure S3

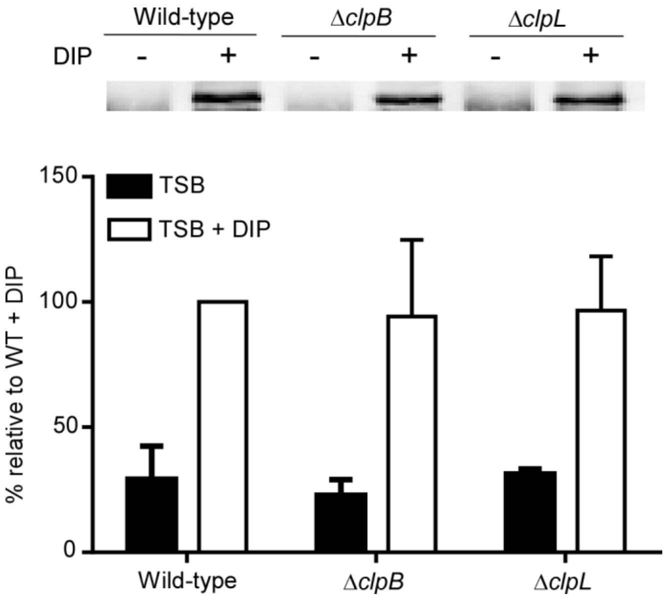

Figure S3. IsdB abundance is unaffected by inactivation of *clpB* or *clpL*. Wild-type *S. aureus* or strains inactivated for *clpB or clpL* were grown in TSB or TSB supplemented with the iron chelator DIP (1 mM). Total protein in cell wall fractions was normalized and separated by 15% SDS-PAGE. Proteins were transferred to nitrocellulose and probed with antibodies directed to IsdB. Blots are representative of 3 independent experiments (*above*). Graphical representation of Isd protein abundance assessed by densitometry in TSB (black bars) or TSB + DIP (white bars) from immunoblot analysis (*below*). Error bars represent SEM.

Farrand *et al.* Figure S4

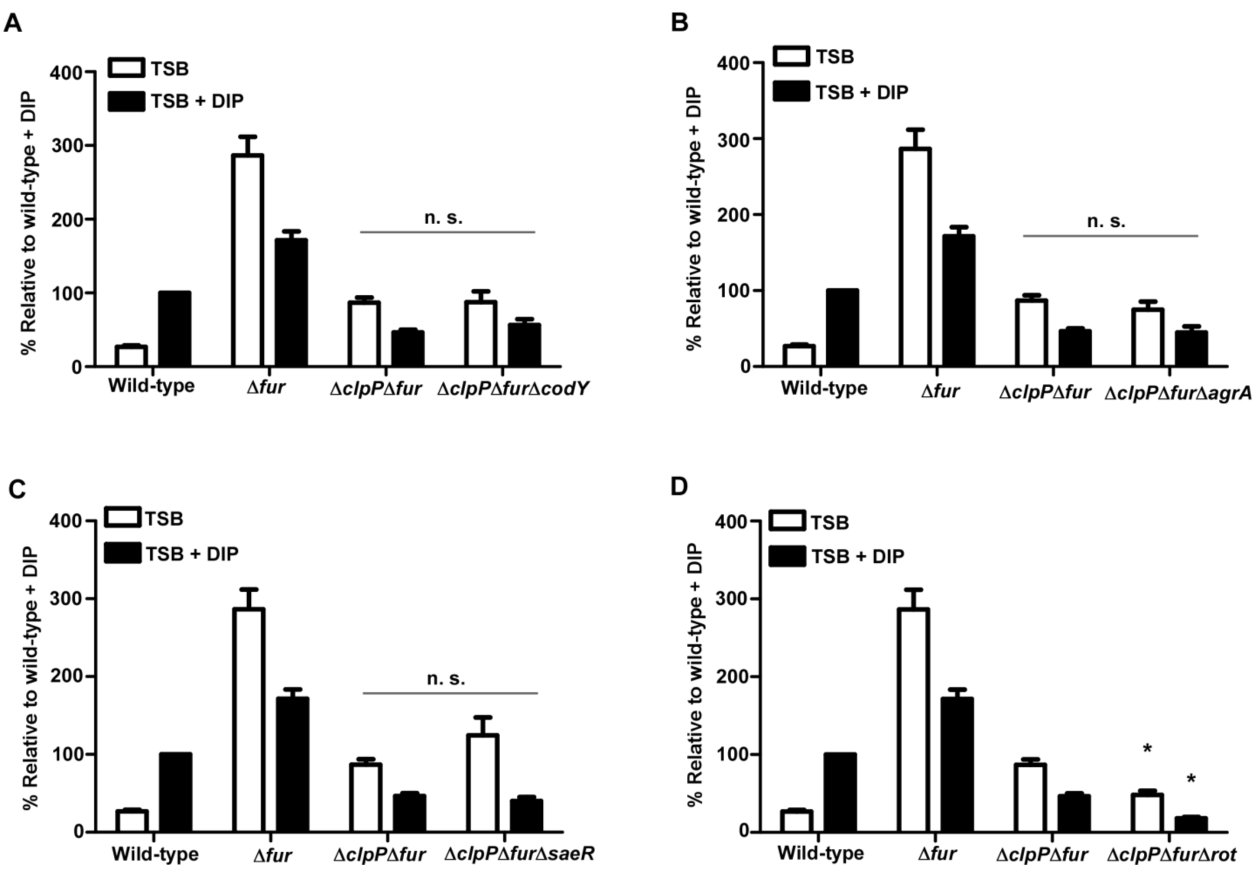

Figure S4. Staphylococcal regulators do not impact clp-dependent IsdB regulation. Wild-type *S. aureus* or strains inactivated for *fur, clpP* and *fur,* or *clpP, fur,* and prominent virulence regulators were grown in TSB (white bars) or TSB supplemented with DIP (black bars). IsdB protein levels were determined in cell wall fractions by Western blot. Data include at least three experiments. Error bars represent SEM. Asterisks denote p value < 0.008 compared to the Δ*clpP*Δ*fur* in the corresponding condition.

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Bacterial Strain	Description	Reference
Newman	*S. aureus* clinical isolate	(70)
Δ*clpP*	Isogenic Newman *clpP* knockout	(40)
Δ*clpP* + *clpP*	Stable integration of *clpP* in chromosome of Δ*clpP*	(40)
Δ*ftsH*	Isogenic Newman *ftsH* knockout	(15)
Δ*hslUV*	Isogenic Newman *hslUV* knockout	(15)
Δ*clpC*	Isogenic Newman *clpC* knockout	This study
Δ*clpX*	Isogenic Newman *clpX* knockout	This study
Δ*clpB*	Isogenic Newman *clpB* knockout	This study
Δ*clpL*	Isogenic Newman *clpL* knockout	This study
Δ*fur*	Allelic replacement of *fur* with tetM cassette	(72)
Δ*clpP*Δ*fur*	Transduction of *fur*::tetM into Δ*clpP*	This study
Δ*isdB*	Allelic replacement of *isdB* with ermC	(8)
Newman pOS1 plgt	Newman transformed with pOS1 plgt empty vector	(9)
Δ*clpP*Δ*fur* pOS1 plgt	Δ*clpP*Δ*fur* transformed with pOS1 plgt empty vector	This study
Δ*clpP*Δ*fur* pOS1 plgt *fur*	Δ*clpP*Δ*fur* transformed with pOS1 plgt *fur* overexpression vector	This study
Δ*clpP*Δ*fur* pOS1 plgt *clpP*	Δ*clpP*Δ*fur* transformed with pOS1 plgt *clpP* overexpression vector	This study
Δ*clpP*Δ*fur*Δ*codY*	Transduction of *codY*::ermC into Δ*clpP*Δ*fur*	This study
Δ*clpP*Δ*fur*Δ*agrA*	Transduction of *agrA*::ermC into Δ*clpP*Δ*fur*	This study
Δ*clpP*Δ*fur*Δ*saeR*	Transduction of *saeR*::ermC into Δ*clpP*Δ*fur*	This study
Δ*clpP*Δ*fur*Δ*rot*	Transduction of *rot*ermC into Δ*clpP*Δ*fur*	This study