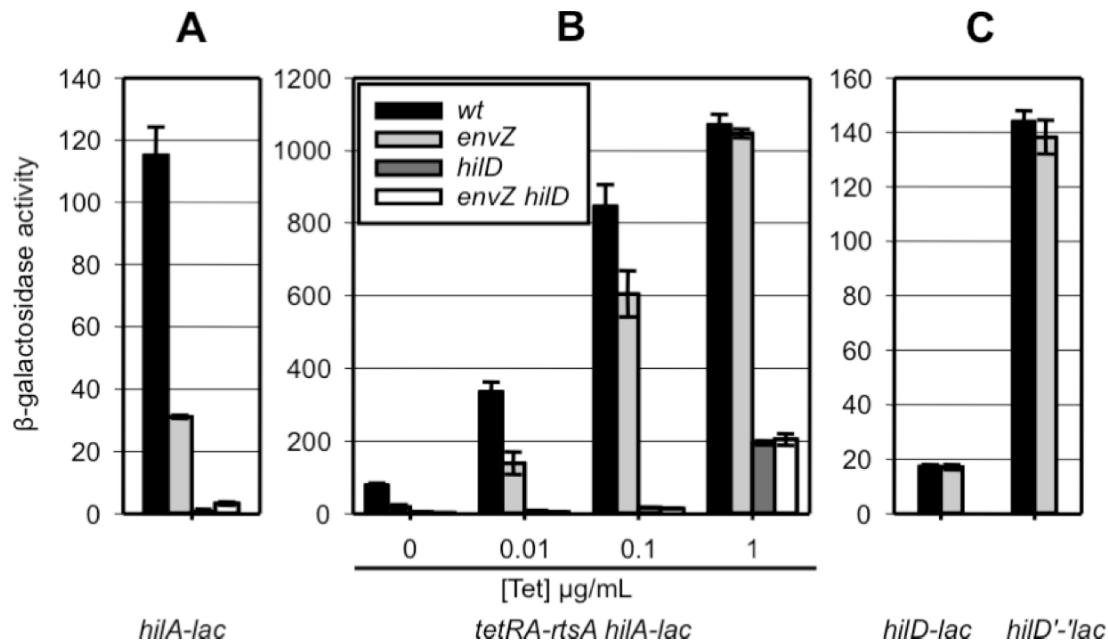
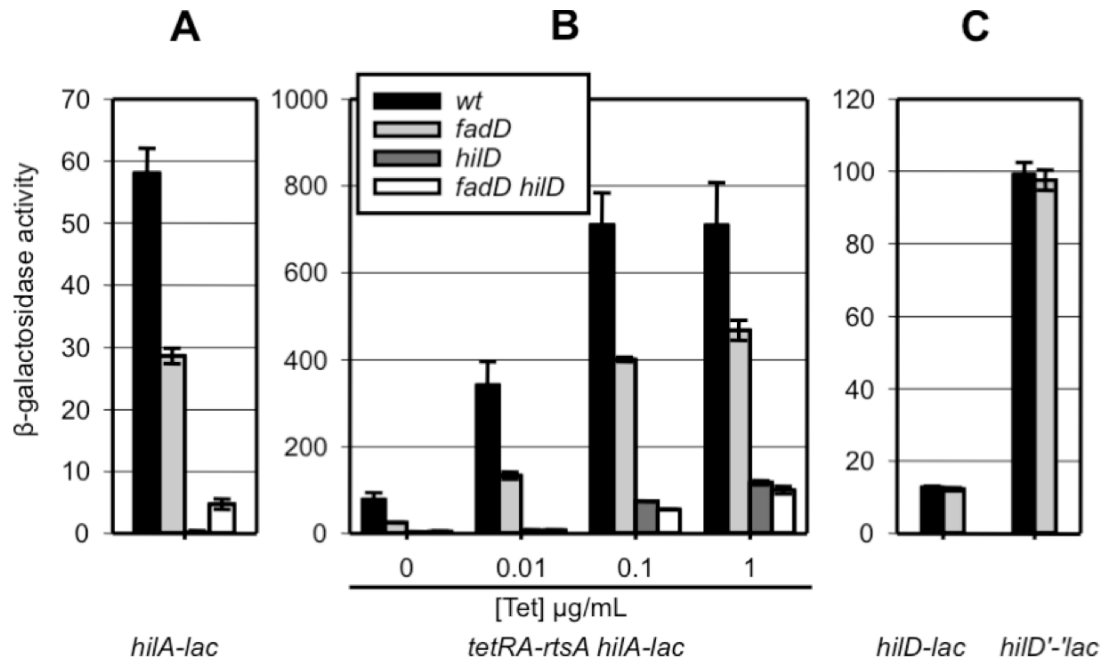


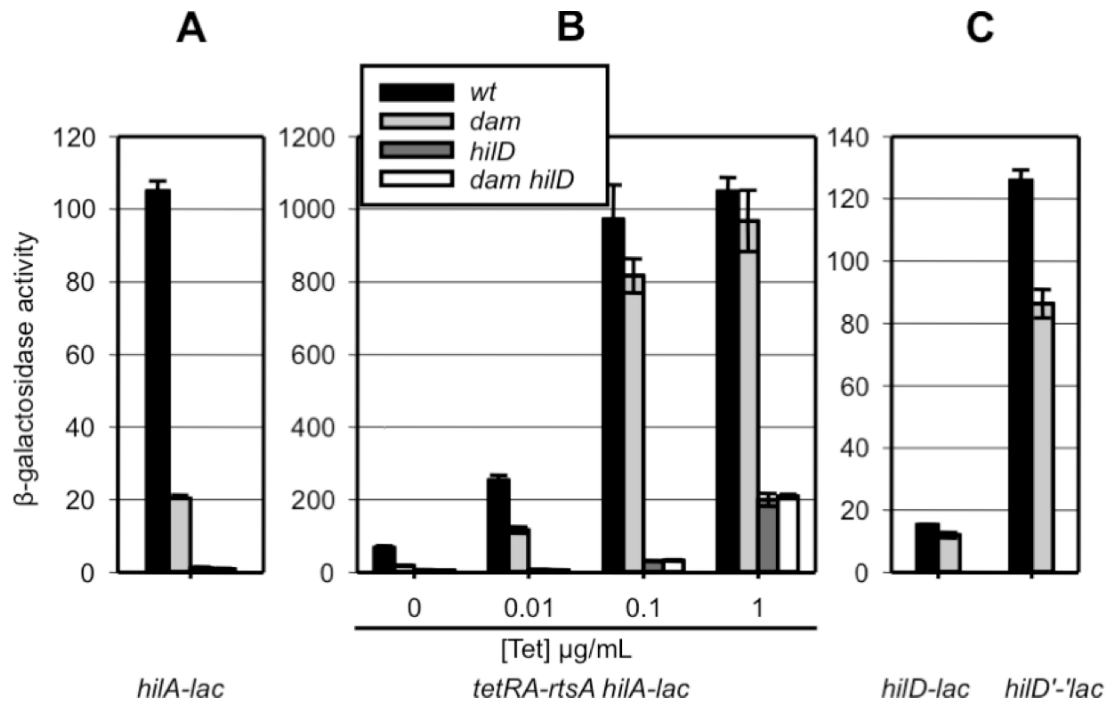
**Figure S1** Class I, FlizZ has no effect on HilD transcription or translation. For the equivalent of panels A and B (for example in Fig. S2) see FIGURE 3 in our previous publication (Chubiz et al., 2010). The panel above (equivalent to panel C in FIGURE S2) shows the  $\beta$ -galactosidase activity in strains containing a *hilD-lac* transcriptional or a *hilD'-lac* translational fusion and the indicated mutations after growth under SPI1 inducing conditions.  $\beta$ -galactosidase activity units are defined as ( $\mu\text{mol of ONP formed min}^{-1}$ )  $\times 10^3 / (\text{OD}_{600} \times \text{ml of cell suspension})$  and are reported as mean  $\pm$  standard deviation where  $n=4$ .



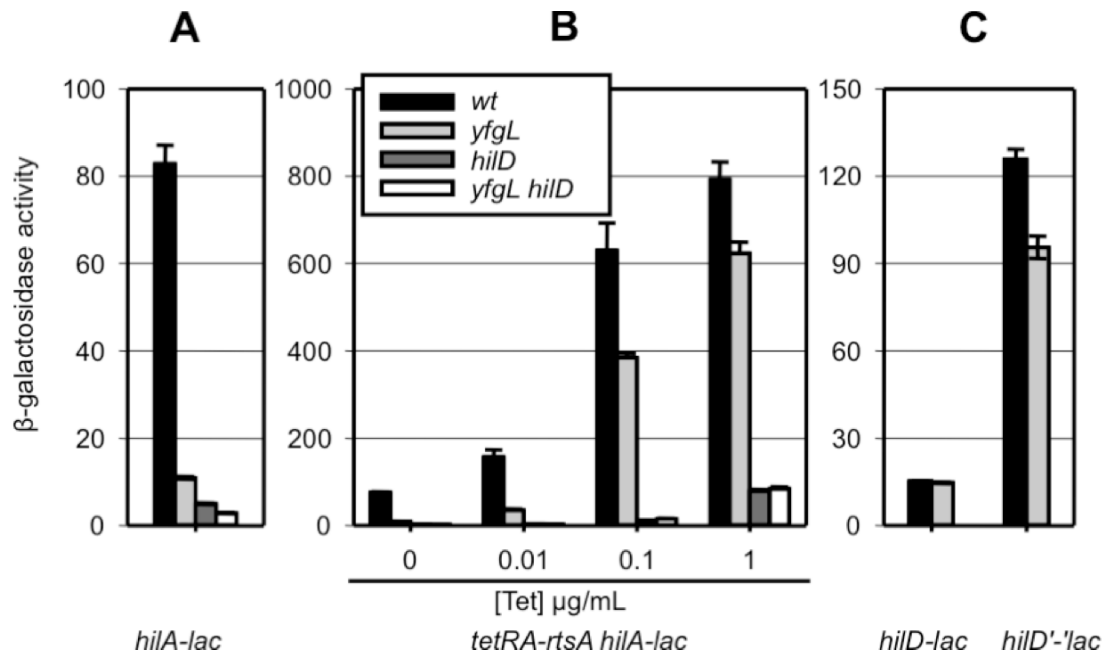
**Figure S2** Class I, EnvZ activates *hilA* expression via post-translational control of HilD. (A)  $\beta$ -galactosidase activity in strains containing a *hilA-lac* transcriptional fusion and the indicated mutations after growth under SPI1 inducing conditions. (B)  $\beta$ -galactosidase activity of strains containing a *hilA-lac* transcriptional fusion and indicated mutations with *rtsA* under the control of a tetracycline regulated promoter. Strains were grown under SPI1-inducing conditions with the indicated tetracycline concentrations. (C)  $\beta$ -galactosidase activity in strains containing a *hilD-lac* transcriptional or a *hilD'-lac* translational fusion and the indicated mutations after growth under SPI1 inducing conditions.  $\beta$ -galactosidase activity units are defined as  $(\mu\text{mol of ONP formed min}^{-1}) \times 10^3 / (\text{OD}_{600} \times \text{ml of cell suspension})$  and are reported as mean  $\pm$  standard deviation where  $n=4$ .



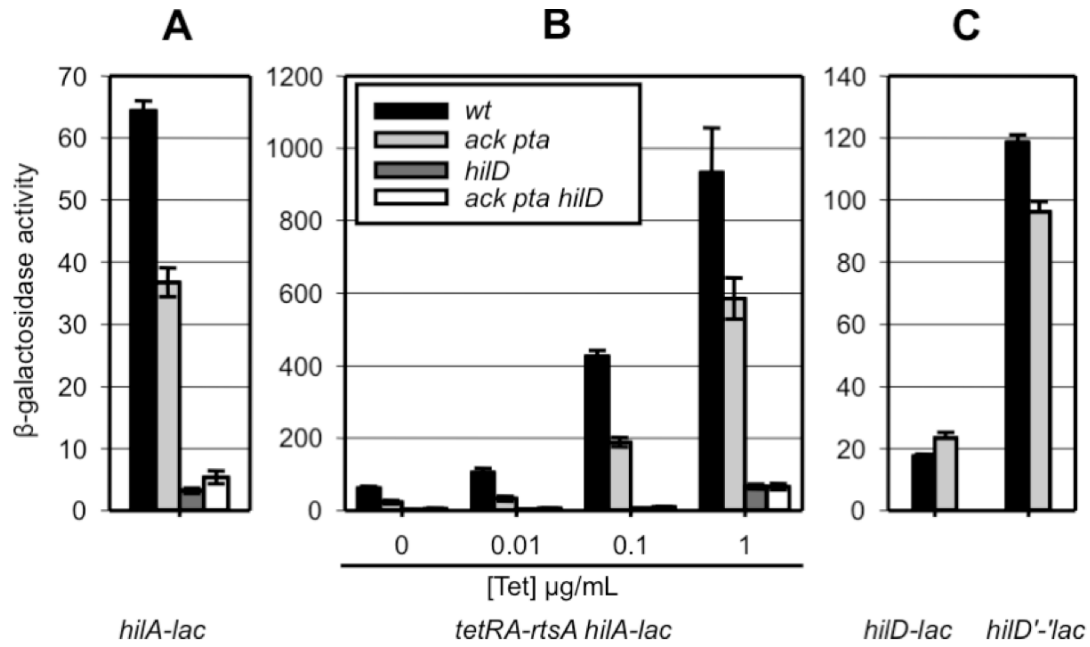
**Figure S3** Class I, FadD activates *hilA* expression via the post-translational control of HiID. See FIGURE S2 legend for details.



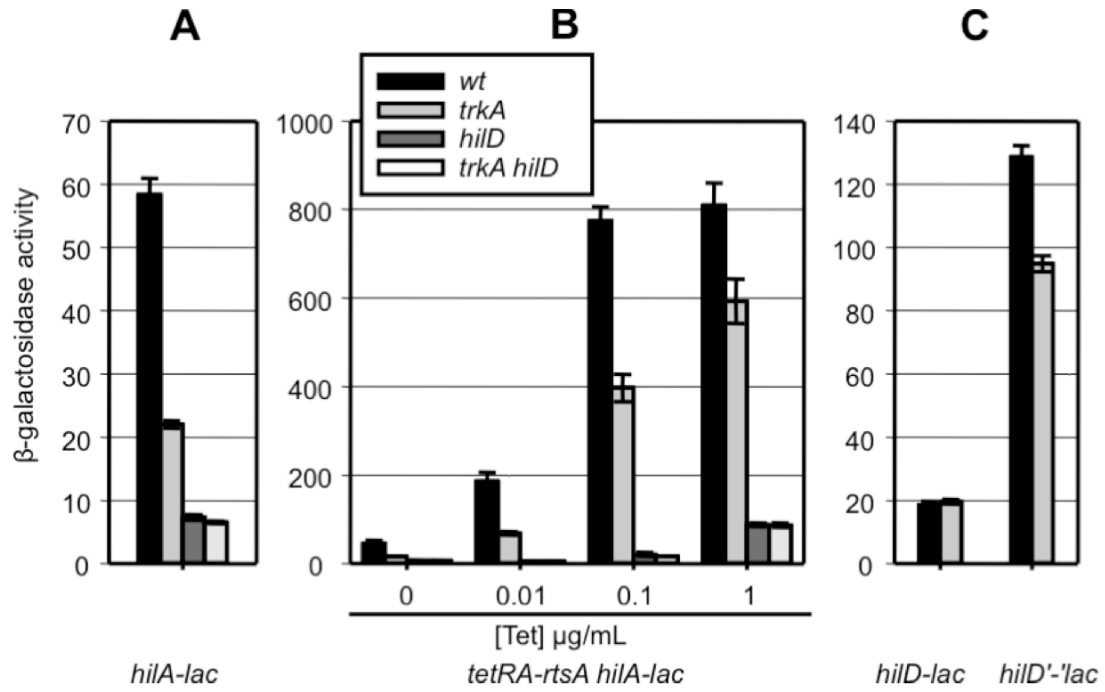
**Figure S4** Class II, Dam activates *hilA* expression via the post-transcriptional control of *hilD*. See FIGURE S2 legend for details.



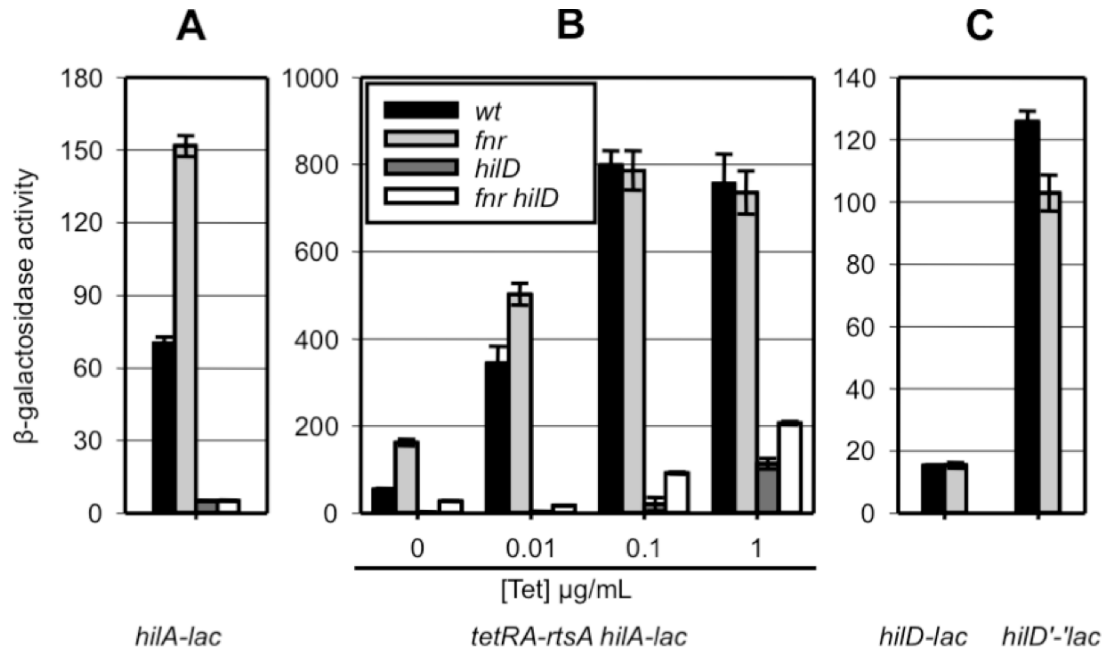
**Figure S5** Class II, YfgL activates *hiA* expression via the post-transcriptional control of *hiD*. See FIGURE S2 legend for details.



**Figure S6** Class II, Ack Pta activates *hilA* expression via the post-transcriptional control of *hilD*. See FIGURE S2 legend for details.

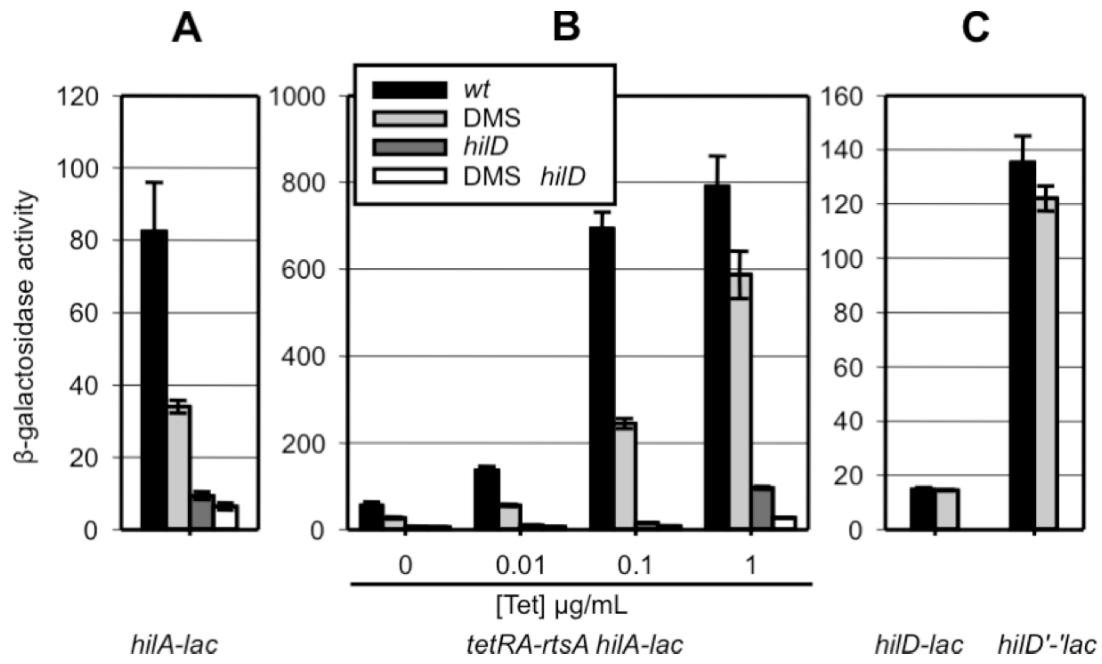


**Figure S7** Class II, TrkA activates *hilA* expression via the post-transcriptional control of *hilD*. See FIGURE S2 legend for details.

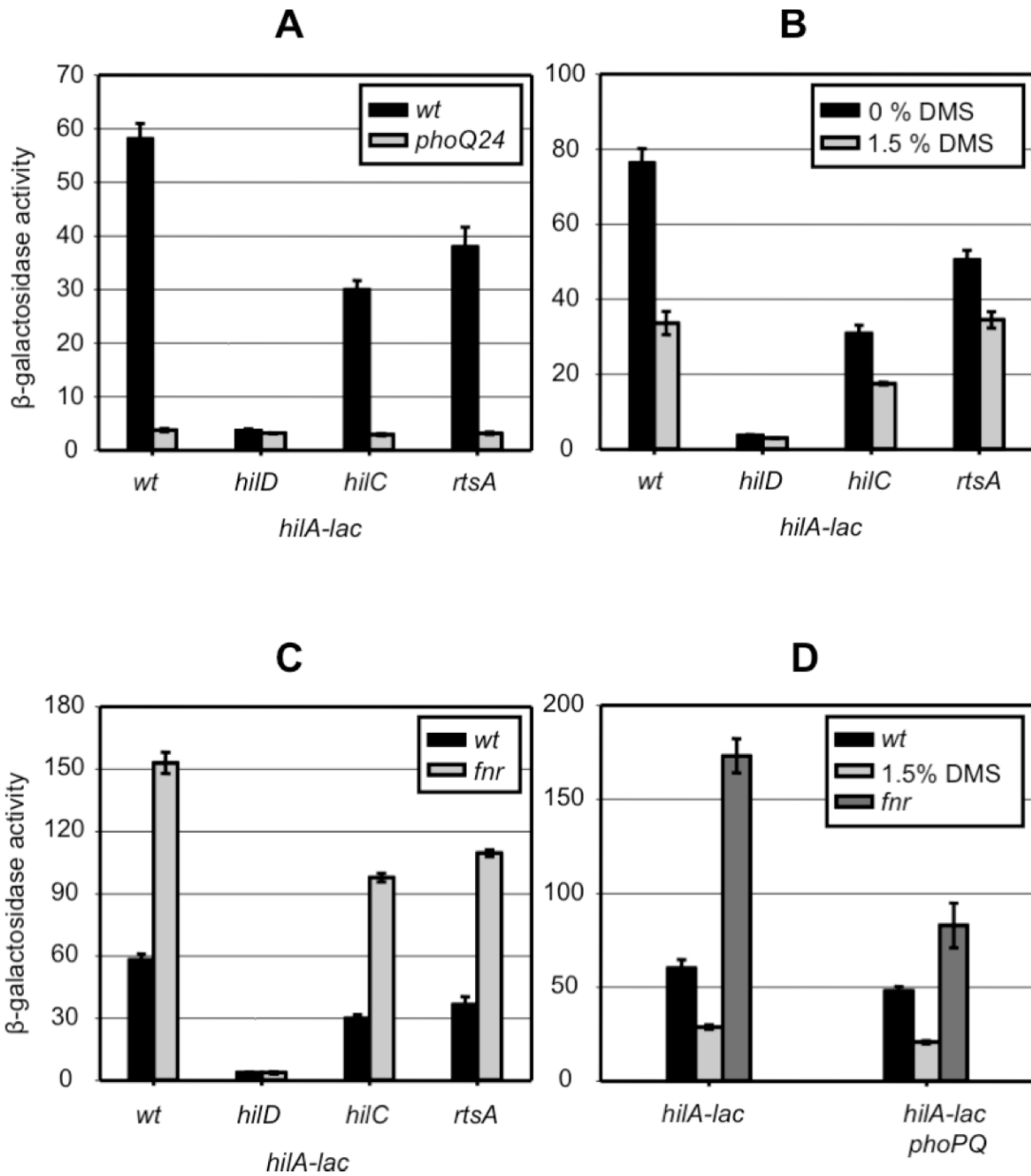


**Figure S8** Class III, Fnr represses *hilA* expression independently of HilD. See FIGURE S2 legend for details.

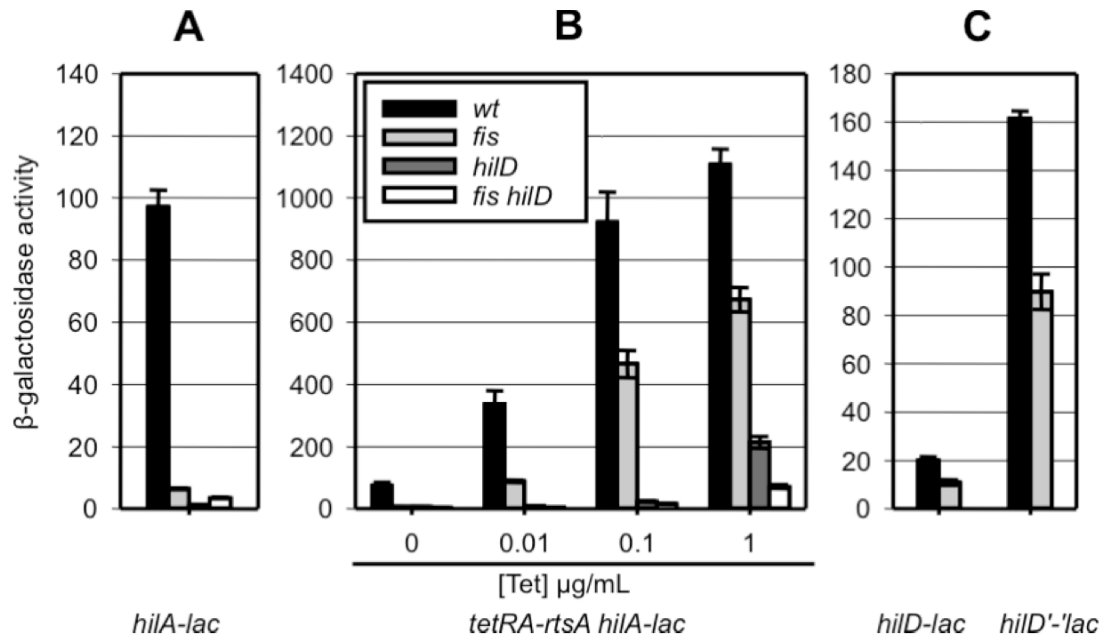




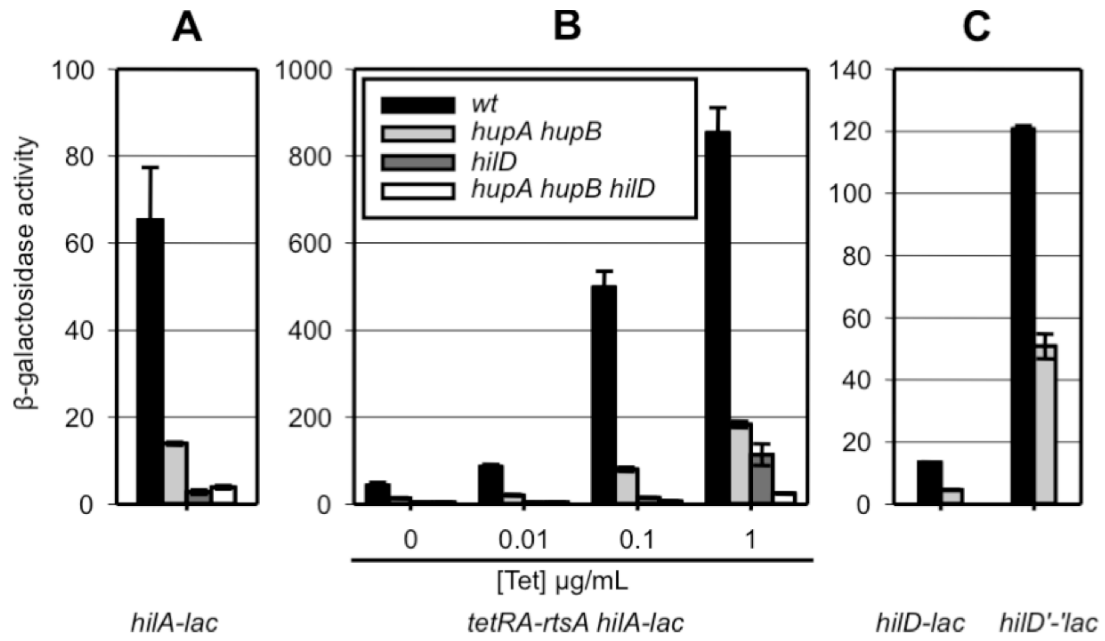
**Figure S9** Class III, Dimethyl sulfide (DMS) represses *hilA* expression independently of HilD. See FIGURE S2 legend for details.



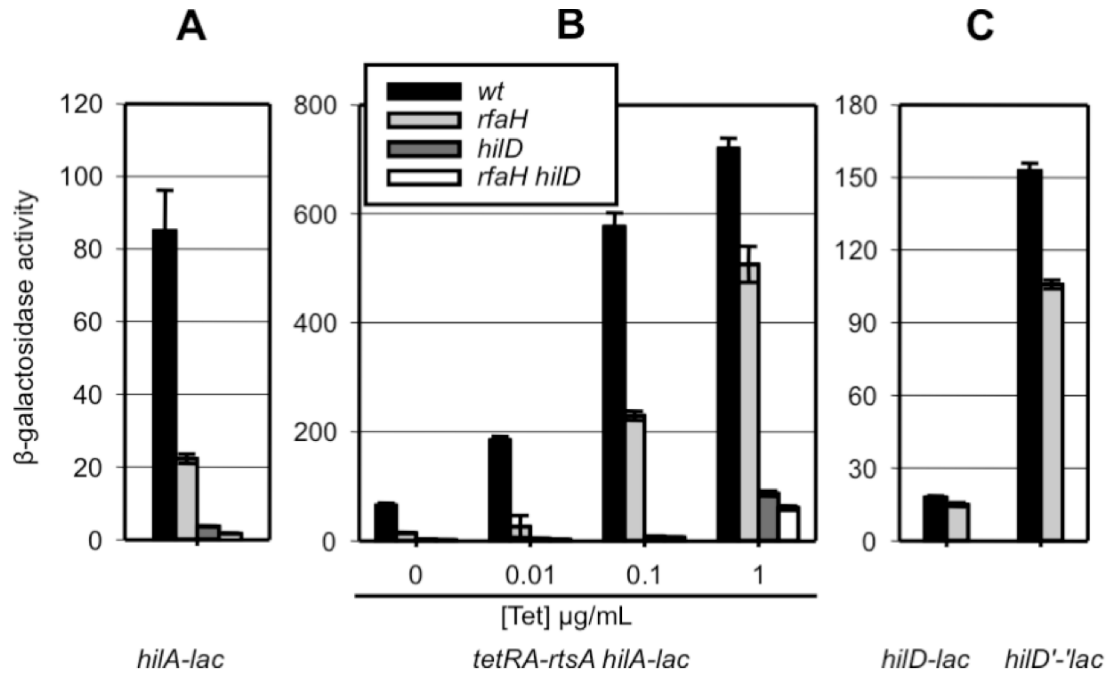
**Figure S10** A) PhoPQ, (B) dimethyl sulfide (DMS), and (C) Fnr repress *hilA* expression independently of HilC and RtsA. (D) Dimethyl sulfide (DMS) and Fnr repress *hilA* expression independently of PhoPQ.  $\beta$ -galactosidase activity in strains containing a *hilA-lac* transcriptional fusion and the indicated mutations, or in the presence or absence of the 1.5% dimethyl sulfide, after growth under SPI1 inducing conditions.  $\beta$ -galactosidase activity units are defined as  $(\mu\text{mol of ONP formed min}^{-1}) \times 10^3 / (\text{OD600} \times \text{ml of cell suspension})$  and are reported as mean  $\pm$  standard deviation where  $n=4$ .



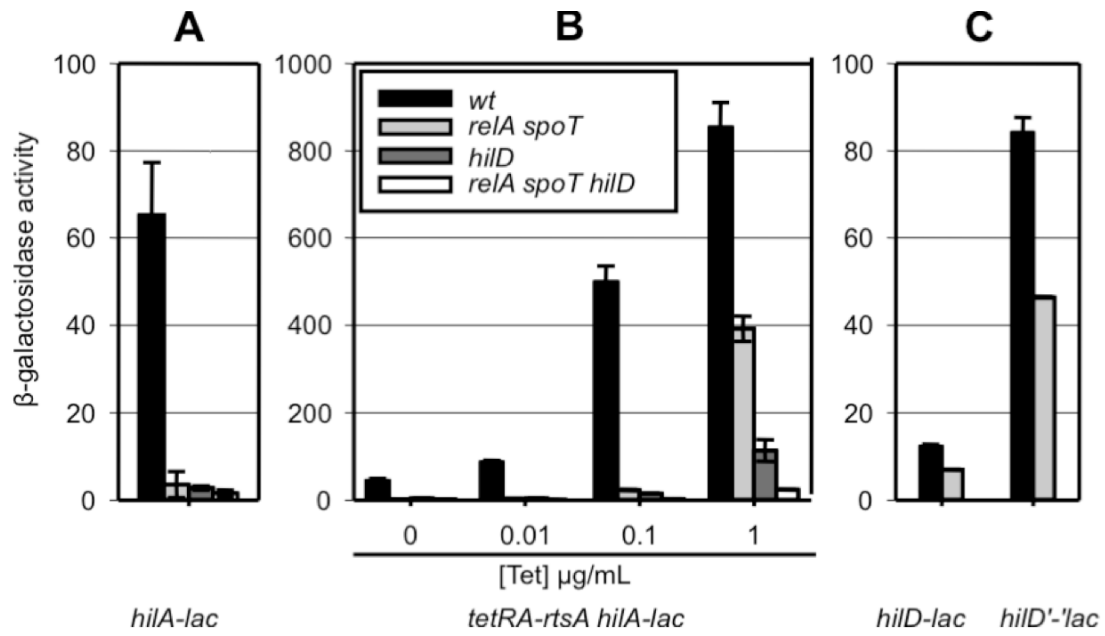
**Figure S11** Class IV, Fis activates SPI 1 expression independently of HlID (affect all promoters in the feed-forward loop). See FIGURE S2 legend for details.



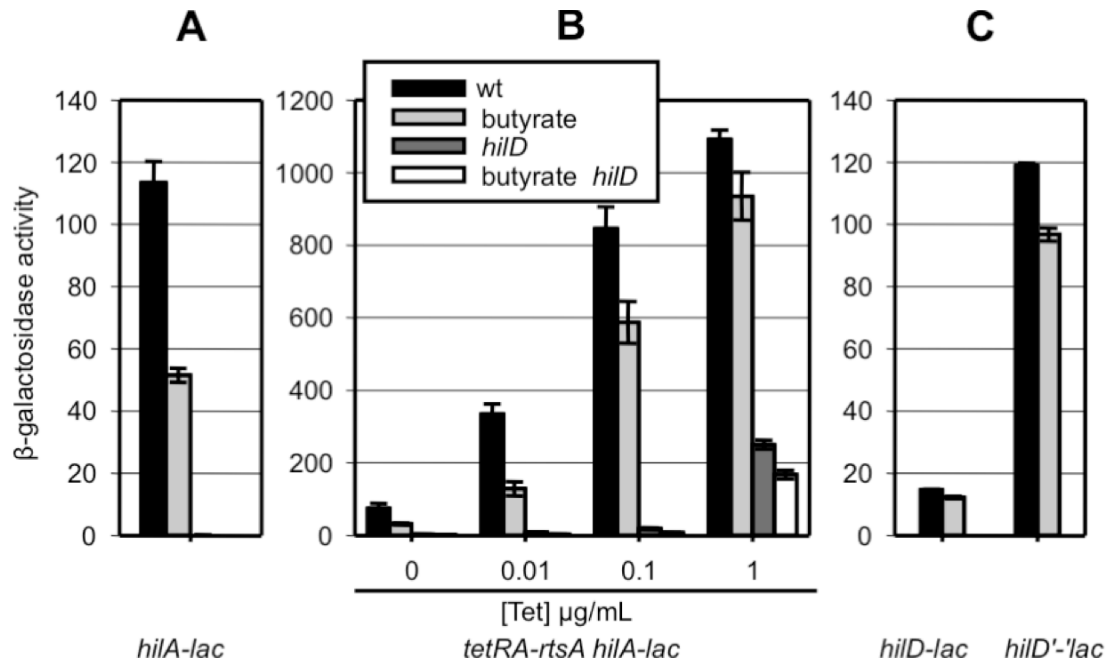
**Figure S12** Class IV, HU (encoded by *hupA hupB*) activates SPI 1 expression independently of HIL D (affect all promoters in the feed-forward loop). See FIGURE S2 legend for details.



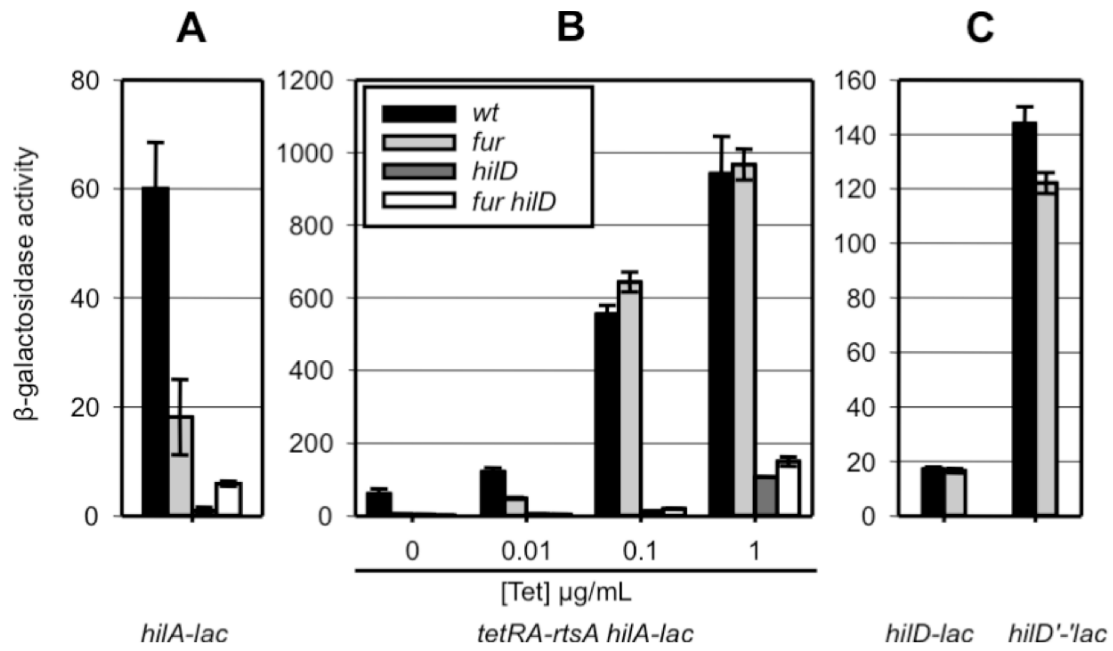
**Figure S13** Class IV, RfaH activates SPI 1 expression independently of HilD (affect all promoters in the feed-forward loop). See FIGURE S2 legend for details.



**Figure S14** Class IV, ppGpp (produced by RelA and SpoT) activates SPI 1 expression independently of HilD (affect all promoters in the feed-forward loop). See FIGURE S2 legend for details.

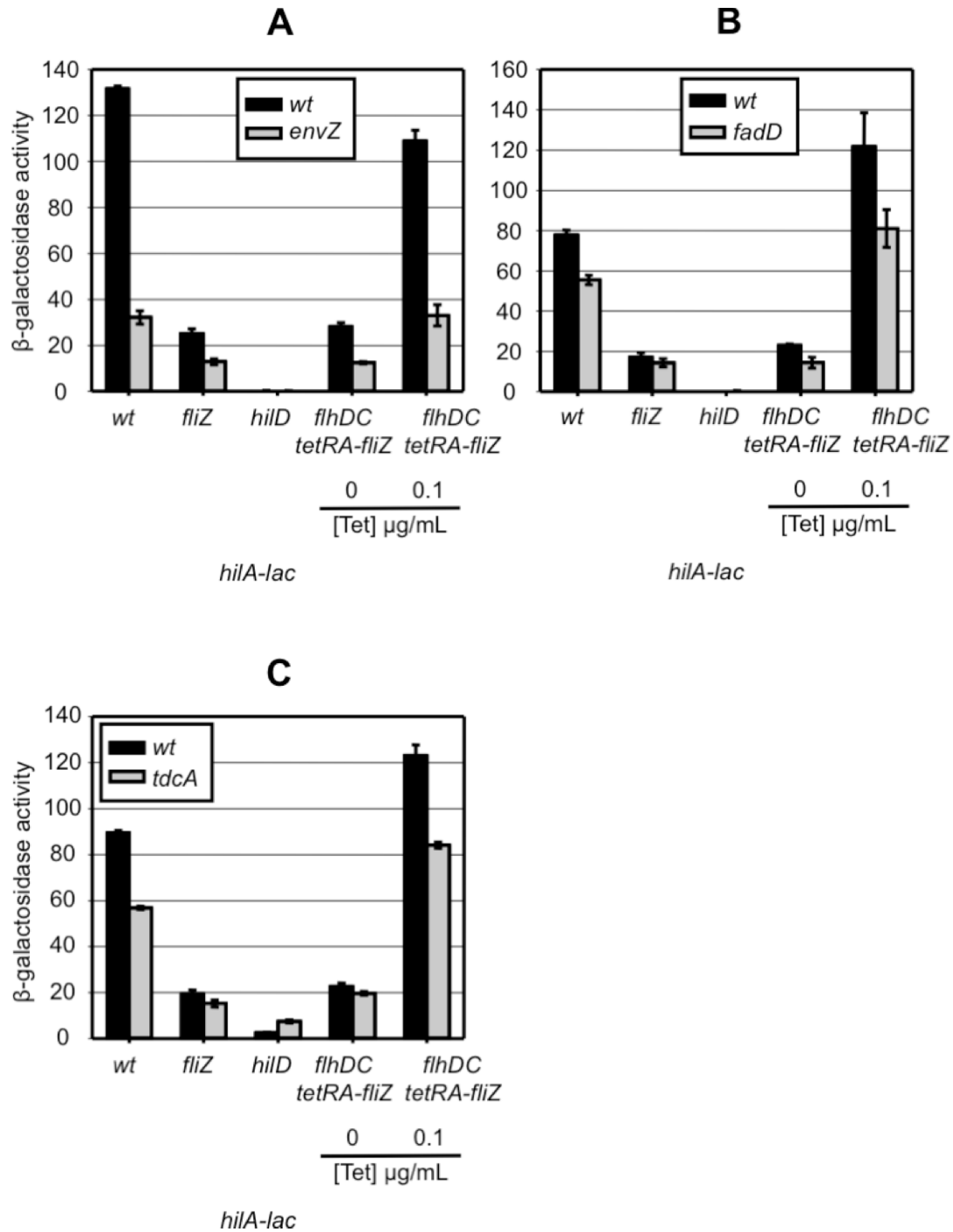


**Figure S15** Class IV, Butyrate represses SPI1 expression independently of HiID (affect all promoters in the feed-forward loop). See FIGURE S2 legend for details.



**Figure S16** Class V, Fur activates *hilA* expression via HilD. See FIGURE S2 legend for details.





**Figure S17** A) EnvZ, (B) FadD, and (C) TdcA affect *hilA* expression independently of *FliZ*.  $\beta$ -galactosidase activity in strains containing a *hilA-lac* transcriptional fusion and the indicated mutations after growth under SPI1 inducing conditions.  $\beta$ -galactosidase activity units are defined as  $(\mu\text{mol of ONP formed min}^{-1}) \times 10^3 / (\text{OD600} \times \text{ml of cell suspension})$  and are reported as mean  $\pm$  standard deviation where  $n=4$ .

**TABLE S1 Regulatory factors/conditions affecting expression of SPI1**

| <b>Regulator</b> | <b>Description</b>   | <b>Mechanism of action/comments</b>  | <b>References</b>   |
|------------------|--|--|---|
| HilA             | Transcriptional activator, OmpR/ToxR family  | Direct activation of <i>prg/org</i> and <i>inv/spa</i> operons                                       | (8; 23; 27; 56; 57)                                       |
| HilD             | AraC-like transcriptional activator  | Direct activation of <i>hilA</i> , <i>hilD</i> , <i>hilC</i> , and <i>rtsA</i>                       | (29; 88)  |
| HilC             | AraC-like transcriptional activator  | Direct activation of <i>hilA</i> , <i>hilD</i> , <i>hilC</i> , and <i>rtsA</i>                       | (28; 29; 46; 88)  |
| RtsA             | AraC-like transcriptional activator  | Direct activation of <i>hilA</i> , <i>hilD</i> , <i>hilC</i> , and <i>rtsA</i>                       | (29; 30)  |
| HilE             |  | Repression of <i>hilA</i> by binding to and preventing HilD function                                 | (11); Chubiz JE (unpublished)                             |
| FlhZ             | Enhancer of class II flagellar genes expression  | Activation of <i>hilA</i> via post-translational regulation of HilD                                  | (20; 45; 54; 59; 85; 102)                                 |
| EnvZ/OmpR        | Two-component regulatory system; regulation of outer membrane porin genes, and virulence   | Activation of <i>hilA</i> via HilD   | (29; 58)  |
| FadD             | Acyl-CoA synthetase; degradation of long-chain fatty acids   | Activation of <i>hilA</i> via an unknown mechanism   | (59); Ellermeier JR (unpublished)                         |
| SirA             | Transcriptional regulator; two-component regulatory system BarA/SirA; regulation of carbohydrate metabolism, motility, biofilm formation, and invasion | Activation of <i>hilA</i> via activation of <i>csrB/csrC</i> to block CsrA repression of <i>hilD</i> | (1; 29; 46; 52; 62; 99; 102); Ellermeier JR (unpublished) |
| Dam              | DNA methylase  | Activation of <i>hilA</i> via post-transcriptional regulation of <i>hilD</i>                         | (55)  |
| Ack Pta          | acetate kinase and phosphotransacetylase   | Activation of <i>hilA</i> and <i>hilD</i> by formate via an unknown mechanism                        | (43)  |

**TABLE S1 Regulatory factors/conditions affecting expression of SPI1**

| <b>Regulator</b> | <b>Description</b>   | <b>Mechanism of action/comments</b>  | <b>References</b>                                 |
|------------------|--|--|---|
| YfgL             | Outer membrane lipoprotein; assembly of the outer membrane $\beta$ -barrel proteins in complex with YaeT, YfiO, and NlpB | Activation of SPI1 genes via an unknown mechanism  | (2; 37)   |
| Trk (potassium)  | Potassium transporter  | Activation of SPI1 genes via an unknown mechanism  | (94)  |
| Fnr              | Transcriptional regulator; cytoplasmic oxygen sensor   | Repression of <i>hilA</i> via an unknown mechanism   | (102); Ellermeier JR (unpublished)                |
| PhoPQ (PhoQ24)   | Two-component regulatory system; response to divalent cation limitation, pH and antimicrobial peptides                   | Repression of <i>hilA</i> , most likely direct   | (9; 10; 14; 78; 111); Ellermeier JR (unpublished) |
| H-NS             | Nucleoid protein   | Direct repression of <i>hilA</i> , <i>hilC</i> , <i>hilD</i> and <i>rtsA</i>   | (73; 74; 89)                                      |
| Hha              | Nucleoid protein   | Direct repression of <i>hilA</i> , <i>hilC</i> , <i>hilD</i> and <i>rtsA</i>   | (36; 73; 74; 102)                                 |
| Fis              | Nucleoid protein   | Activation of SPI1 genes   | (22; 48; 89; 110)}                                |
| HU               | Nucleoid protein   | Activation of SPI1 genes   | (60; 89)  |
| RfaH             | Transcriptional anti-terminator; long operons for LPS core and O-antigen biosynthesis                                    | Activation of SPI1 genes via an unknown mechanism  | (60; 67)  |
| Fur              | Transcriptional regulator; response to iron  | Activation of <i>hilA</i> via an unknown regulation of HilD; repression of H-NS; direct binding of Fur to <i>hilD</i> promoter | (32; 98; 101)                                     |
| TdcA             | Transcriptional regulator of <i>tdc</i> operon; transport and metabolism of L-   | Activation of <i>fliZ</i> and SPI1 genes expression  | (50)  |

**TABLE S1 Regulatory factors/conditions affecting expression of SPI1**

| Regulator              | Description  | Mechanism of action/comments   | References                                    |
|------------------------|--|--|---|
|                        | threonine and L-serine   |  |   |
| FhDC                   | Transcriptional regulator; activation of class II flagellar genes  | Activation of <i>hilA</i> via activation of <i>fliZ</i>  | (20; 54)                                      |
| DsbA                   | Periplasmic disulfide bond oxidase   | Activation of <i>hilA</i> via activation of <i>FliZ</i> , including repression of <i>RcsCDB</i>              | (31; 54)                                      |
| RcsCDB                 | phosphorelay system: sensor <i>RcsC</i> , response regulator <i>RcsB</i> , and phosphotransfer protein <i>RcsD</i> ; regulation of capsule synthesis and biofilm formation | Repression of <i>hilA</i> via repression of <i>FliZ</i> and an independent unknown regulation of <i>HilD</i> | (54)  |
| Lon                    | ATP-dependent protease   | Repression of <i>hilA</i> via degradation of <i>HilD</i> , <i>HilC</i> , and <i>FliZ</i>                     | (16; 20; 95; 97)                              |
| ClpXP                  | ATP-dependent protease   | Repression of SPI1 genes via <i>FliZ</i>   | (47)  |
| Formate                | Short chain fatty acid   | Activation of <i>hilA</i> and <i>hilD</i> ( see Ack Pta)   | (43)  |
| Dimethyl sulfide/ DMSO |  | Repression of SPI1 genes via an unknown mechanism  | (4)   |
| Temperature            |  | Activation of SPI1 genes when shifted from 25° to 37°C in H-NS-dependent manner                              | (75)  |
| Butyrate               | Short chain fatty acid   | Repression of SPI1 genes via an unknown mechanism  | (41)  |
| ppGpp                  | Small signaling molecule; stringent response during starvation   | Activation of <i>hilA</i> via an unknown mechanism   | (79; 92; 93; 100);Ellermeier JR (unpublished) |

**TABLE S1 Regulatory factors/conditions affecting expression of SPI1**

| <b>Regulator</b> | <b>Description</b>   | <b>Mechanism of action/comments</b>  | <b>References</b> |
|------------------|--|--|-------------------|
| FimZY            | Transcriptional regulators; control of type 1 fimbriae gene expression   | Repression of <i>hilA</i> via activation of <i>hilE</i>                            | (12; 87)          |
| FimW             | negative regulator of type 1 fimbriae  | Activation of SPI1 genes via an unknown mechanism                                  | (38)              |
| Mlc              | Transcriptional regulator; regulation of sugar uptake and metabolism   | Repression of <i>hilA</i> via activation of <i>hilE</i>                            | (53)              |
| CRP              | cAMP-receptor protein  | Activation of invasion via an unknown mechanism                                    | (17)              |
| CpxA             | Sensor kinase, two-component regulatory system CpxRA; periplasmic stress response  | Activation of <i>hilA</i> via an unknown mechanism, apparently independent of CpxR | (68)              |
| Lrp              | Transcriptional regulator  | Overproduction of Lrp represses SPI1 genes via an unknown mechanism                | (6)               |
| PmrM             | Part of the <i>pmrHFIJKLM</i> operon   | Activation of <i>hilA</i> via an unknown mechanism                                 | (61; 65; 102)     |
| ApaH/YgdP        | Dinucleoside polyphosphate hydrolases  | Activation of invasion via an unknown mechanism                                    | (44)              |
| PreAB (QseBC)    | Two-component regulatory system; regulation of motility and virulence in response to quorum-sensing and hormonal signals | Activation of SPI1 genes via an unknown mechanism                                  | (64; 66)          |
| LuxS             | Autoinducer 2 synthase   | Activation of <i>invF</i>  | (19)              |
| PhoBR            | Two-component regulatory system; phosphate limitation  | Repression of <i>hilA</i> via PhoBR  | (59)              |

**TABLE S1 Regulatory factors/conditions affecting expression of SPI1**

| <b>Regulator</b> | <b>Description</b>   | <b>Mechanism of action/comments</b>                                   | <b>References</b> |
|------------------|--|---|-------------------|
| SprB             | Transcriptional regulator  | Repression of <i>hilA</i> via repression of <i>hilD</i> transcription | (86)              |
| RamA             | AraC/XylS family transcriptional activator; regulation of multidrug resistance | Repression of SPI1 genes via an unknown mechanism                     | (7)               |
| PPK              | Polyphosphate kinase   | Activation of invasion via an unknown mechanism                       | (49)              |
| PNPase           | Polynucleotide phosphorylase   | Repression of SPI1 genes via an unknown mechanism                     | (21)              |
| Hfq              | RNA shaperone  | Activation of SPI1 genes  | (3; 90; 91)       |
| SmpB             | RNA-binding protein  | Activation of SPI1 genes  | (3)               |
| IHF              | Nucleoid protein   | Activation of <i>hilA</i> ; counteraction of H-NS mediated silencing  | (35; 83)          |
| RNAseE           | 5'-end-dependent endoribonuclease; part of degradosome complex                 | Repression of SPI1 genes; exact mechanism unclear                     | (35)              |
| Pag              |  | Repression of SPI1 genes via an unknown mechanism                     | (35)              |
| SirB             |  | Activation of <i>hilA</i> ; exact mechanism unclear                   | (84)              |
| Sig32            | Sigma factor; heat shock response  | Repression of <i>hilA</i> via degradation of HilD by Lon protease     | (63)              |
| ToIC/AcrAB       | Multidrug efflux pump  | Activation of SPI1 genes via an unknown mechanism                     | (15; 103; 107)    |
| AsmA             | Outer membrane protein   | Required for invasion; unknown mechanism                              | (80)              |
| CorA             | Mg <sup>2+</sup> channel   | Activation of SPI1 genes via an unknown mechanism                     | (76; 77)          |

**TABLE S1** Regulatory factors/conditions affecting expression of SPI1

| <b>Regulator</b>                      | <b>Description</b>                              | <b>Mechanism of action/comments</b>                | <b>References</b> |
|---------------------------------------|---|--|-------------------|
| PoxA                                  | paralog of lysyl tRNA-synthetase                | Repression of SPI1 genes via an unknown mechanism  | (70)              |
| YjeK                                  | putative 2,3- $\beta$ -lysine aminomutase       | Repression of SPI1 genes via an unknown mechanism  | (70)              |
| Antimicrobial peptides                |   | Repression of SPI1 genes via PhoPQ                 | (5)               |
| Macrophages                           |   | Repression of SPI1 genes                           | (33; 96)          |
| Epithelial cells                      |   | Activation of SPI1 genes                           | (42)              |
| Bile                                  | Role in lipid digestion                         | Repression of <i>hilA</i> via SirA                 | (81; 82)          |
| Propanediol                           | Product of decomposition of rhamnose and fucose | Repression of <i>hilA</i> via an unknown mechanism | (69)              |
| Microgravity                          | low-shear modeled microgravity                  | Repression of SPI1 genes                           | (109)             |
| Lactobacillus supernatant; probiotics |   | Repression of SPI1 genes via an unknown mechanism  | (25); (13)        |
| Tetracycline                          |   | Activation of SPI1 genes                           | (108)             |
| Nalidixic acid                        |   | Repression of SPI1 genes                           | (26)              |
| Salicylidene acylhydrazides           |   | Repression of SPI1 genes                           | (71)              |
| Fluoroquinolone resistance            |   | Repression of SPI1 genes                           | (34)              |

**TABLE S2 Strains and plasmids**

| <b>Name</b> | <b>Genotype<sup>a</sup></b>  | <b>Deletion endpoints<sup>b</sup></b> | <b>Source or reference<sup>c</sup></b> |
|-------------|--|---------------------------------------|--|
| 14028       | Wild type  |                                       | ATCC <sup>d</sup>                      |
| JS564       | <i>ΔhilD138::Kn</i>  |                                       | (32)                                   |
| JS253       | <i>ΔhilD114::Cm</i>  |                                       | (30)                                   |
| JS749       | <i>attλ::pDX1::hilA'-lacZ</i>                                      |                                       | (54)                                   |
| JS951       | <i>ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>                         |                                       | (20)                                   |
| JS576       | <i>ΔhilD114::Cm attλ::pDX1::hilA'-lacZ</i>                         |                                       | (32)                                   |
| JS953       | <i>tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>                           |                                       | (20)                                   |
| JS955       | <i>ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>              |                                       | (20)                                   |
| JS488       | $\Phi(hilD'-lac^+)114$   |                                       |  |
| JS892       | $\Phi(hilD'-lacZ)hyb139$   |                                       | (20)                                   |
| JS996       | <i>ΔhilE115::Cm</i>  | 4763527-<br>4764108                   |  |
| JS997       | <i>ΔhilE115::Cm attλ::pDX1::hilA'-lacZ</i>                         |                                       |  |
| JS998       | <i>ΔhilE115::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>            |                                       |  |
| JS999       | <i>ΔhilE115::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>              |                                       |  |
| JS1000      | <i>ΔhilE115::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i> |                                       |  |
| JS1001      | $\Phi(hilD'-lac^+)114 \Delta hilE115::Cm$                          |                                       |  |
| JS1002      | $\Phi(hilD'-lacZ)hyb139 \Delta hilE115::Cm$                        |                                       |  |
| JS950       | <i>ΔfliZ8042::Cm attλ::pDX1::hilA'-lacZ</i>                        |                                       | (20)                                   |
| JS1003      | $\Phi(hilD'-lac^+)114 \Delta fliZ8042::Cm$                         |                                       |  |
| JS1004      | $\Phi(hilD'-lacZ)hyb139 \Delta fliZ8042::Cm$                       |                                       |  |
| JS1006      | <i>ΔenvZ182::Cm attλ::pDX1::hilA'-lacZ</i>                         |                                       |  |
| JS1007      | <i>ΔenvZ182::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>            |                                       |  |
| JS1008      | <i>ΔenvZ182::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>              |                                       |  |
| JS1009      | <i>ΔenvZ182::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i> |                                       |  |
| JS1010      | $\Phi(hilD'-lac^+)114 \Delta envZ182::Cm$                          |                                       |  |
| JS1011      | $\Phi(hilD'-lacZ)hyb139 \Delta envZ182::Cm$                        |                                       |  |
| JS1012      | <i>ΔfadD21::Kn</i>   | 1915235-<br>1916908                   |  |
| JS1013      | <i>ΔfadD21::Kn attλ::pDX1::hilA'-lacZ</i>                          |                                       |  |
| JS1014      | <i>ΔfadD21::Kn ΔhilD114::Cm attλ::pDX1::hilA'-lacZ</i>             |                                       |  |
| JS1015      | <i>ΔfadD21::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>               |                                       |  |
| JS1016      | <i>ΔfadD21::Kn ΔhilD114::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>  |                                       |  |
| JS1017      | $\Phi(hilD'-lac^+)114 \Delta fadD21$                               |                                       |  |
| JS1018      | $\Phi(hilD'-lacZ)hyb139 \Delta fadD21$                             |                                       |  |
| JS1019      | <i>sirA3::Cm attλ::pDX1::hilA'-lacZ</i>                            |                                       |  |



| Name   | Genotype <sup>a</sup>  | Deletion endpoints <sup>b</sup> | Source or reference <sup>c</sup> |
|--------|--|---------------------------------|----------------------------------|
| JS1020 | <i>sirA3::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>                     |                                 |                                  |
| JS1021 | <i>sirA3::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>                       |                                 |                                  |
| JS1022 | <i>sirA3::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>          |                                 |                                  |
| JS1023 | Φ( <i>hilD'-lac<sup>+</sup></i> )114 <i>sirA3::Cm</i>                    |                                 |                                  |
| JS1024 | Φ( <i>hilD'-lacZ</i> ) <i>hybb139 sirA3::Cm</i>                          |                                 |                                  |
| JS1025 | <i>Δdam241::Cm</i>   | 3638689-<br>3639527             |                                  |
| JS1026 | <i>Δdam241::Cm attλ::pDX1::hilA'-lacZ</i>                                |                                 |                                  |
| JS1027 | <i>Δdam241::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>                   |                                 |                                  |
| JS1028 | <i>Δdam241::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>                     |                                 |                                  |
| JS1029 | <i>Δdam241::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>        |                                 |                                  |
| JS1030 | Φ( <i>hilD'-lac<sup>+</sup></i> )114 <i>Δdam241::Cm</i>                  |                                 |                                  |
| JS1031 | Φ( <i>hilD'-lacZ</i> ) <i>hyb139 Δdam241::Cm</i>                         |                                 |                                  |
| JS1032 | <i>Δ(ack-pta)4202::Cm</i>  | 2447938-<br>2451363             |                                  |
| JS1033 | <i>Δ(ack-pta)4202::Cm attλ::pDX1::hilA'-lacZ</i>                         |                                 |                                  |
| JS1034 | <i>Δ(ack-pta)4202::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>            |                                 |                                  |
| JS1035 | <i>Δ(ack-pta)4202::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>              |                                 |                                  |
| JS1036 | <i>Δ(ack-pta)4202::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i> |                                 |                                  |
| JS1037 | Φ( <i>hilD'-lac<sup>+</sup></i> )114 <i>Δ(ack-pta)4202::Cm</i>           |                                 |                                  |
| JS1038 | Φ( <i>hilD'-lacZ</i> ) <i>hyb139 Δ(ack-pta)4202::Cm</i>                  |                                 |                                  |
| JS1180 | <i>ΔtrkA::Cm</i>   | 3579771-<br>3581196             |                                  |
| JS1181 | <i>Δ trkA::Cm attλ::pDX1::hilA'-lacZ</i>                                 |                                 |                                  |
| JS1182 | <i>Δ trkA::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>                    |                                 |                                  |
| JS1183 | <i>Δ trkA::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>                      |                                 |                                  |
| JS1184 | <i>Δ trkA::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>         |                                 |                                  |
| JS1185 | Φ( <i>hilD'-lac<sup>+</sup></i> )114 <i>ΔtrkA::Cm</i>                    |                                 |                                  |
| JS1186 | Φ( <i>hilD'-lacZ</i> ) <i>hyb139 ΔtrkA::Cm</i>                           |                                 |                                  |
| JS1039 | <i>ΔyfgL611::Cm</i>  | 2653048-<br>2654226             |                                  |
| JS1040 | <i>ΔyfgL611::Cm attλ::pDX1::hilA'-lacZ</i>                               |                                 |                                  |
| JS1041 | <i>ΔyfgL611::Cm ΔhilD138::Kn attλ::pDX1::hilA'-lacZ</i>                  |                                 |                                  |
| JS1042 | <i>ΔyfgL611::Cm tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>                    |                                 |                                  |
| JS1043 | <i>ΔyfgL611::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hilA'-lacZ</i>       |                                 |                                  |
| JS1044 | Φ( <i>hilD'-lac<sup>+</sup></i> )114 <i>ΔyfgL611::Cm</i>                 |                                 |                                  |
| JS1045 | Φ( <i>hilD'-lacZ</i> ) <i>hyb139 ΔyfgL611::Cm</i>                        |                                 |                                  |

| Name   | Genotype <sup>a</sup>   | Deletion endpoints <sup>b</sup> | Source or reference <sup>c</sup> |
|--------|---|---------------------------------|----------------------------------|
| JS1046 | <i>Δfnr1::Cm</i>  | 1754380-<br>1755116             |                                  |
| JS1047 | <i>Δfnr1::Cm attλ::pDX1::hila'-lacZ</i>                                   |                                 |                                  |
| JS1048 | <i>Δfnr1::Cm ΔhilD138::Kn attλ::pDX1::hila'-lacZ</i>                      |                                 |                                  |
| JS1049 | <i>Δfnr1::Cm tetRA-rtxA attλ::pDX1::hila'-lacZ</i>                        |                                 |                                  |
| JS1050 | <i>Δfnr1::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hila'-lacZ</i>           |                                 |                                  |
| JS1051 | $\Phi(hilD'-lac^+)$ 114 <i>Δfnr1::Cm</i>                                  |                                 |                                  |
| JS1052 | $\Phi(hilD'-lacZ)hyb139$ <i>Δfnr1::Cm</i>                                 |                                 |                                  |
| JS1053 | <i>ΔycfD612::Kn</i>   | 1316880                         |                                  |
| JS1054 | <i>phoQ24 ΔycfD612::Kn</i>  |                                 |                                  |
| JS1055 | <i>phoQ24 ΔycfD612::Kn attλ::pDX1::hila'-lacZ</i>                         |                                 |                                  |
| JS1056 | <i>phoQ24 ΔycfD612::Kn ΔhilD114::Cm attλ::pDX1::hila'-lacZ</i>            |                                 |                                  |
| JS1057 | <i>phoQ24 ΔycfD612::Kn tetRA-rtxA attλ::pDX1::hila'-lacZ</i>              |                                 |                                  |
| JS1058 | <i>phoQ24 ΔycfD612::Kn ΔhilD114::Cm tetRA-rtxA attλ::pDX1::hila'-lacZ</i> |                                 |                                  |
| JS1059 | $\Phi(hilD'-lac^+)$ 114 <i>phoQ24 ΔycfD612</i>                            |                                 |                                  |
| JS1060 | $\Phi(hilD'-lacZ)hyb139$ <i>phoQ24 ΔycfD612</i>                           |                                 |                                  |
| JS577  | <i>ΔhilC113::Cm attλ::pDX1::hila'-lacZ</i>                                |                                 |                                  |
| JS579  | <i>ΔrtsA5 attλ::pDX1::hila'-lacZ</i>                                      |                                 |                                  |
| JS1061 | <i>phoQ24 ΔycfD612::Kn ΔhilC113::Cm attλ::pDX1::hila'-lacZ</i>            |                                 |                                  |
| JS1062 | <i>phoQ24 ΔycfD612::Kn ΔrtsA5 attλ::pDX1::hila'-lacZ</i>                  |                                 |                                  |
| JS1063 | <i>Δfnr2::Tet</i>   | 1754321-<br>1755129             |                                  |
| JS1064 | <i>Δfnr2::Tet attλ::pDX1::hila'-lacZ</i>                                  |                                 |                                  |
| JS1065 | <i>Δfnr2::Tet ΔhilD114::Cm attλ::pDX1::hila'-lacZ</i>                     |                                 |                                  |
| JS1066 | <i>Δfnr2::Tet ΔhilC113::Cm attλ::pDX1::hila'-lacZ</i>                     |                                 |                                  |
| JS1067 | <i>Δfnr2::Tet ΔrtsA5 attλ::pDX1::hila'-lacZ</i>                           |                                 |                                  |
| JS1068 | <i>ΔphoPQ::Cm</i>   | 1317242-<br>1319310             |                                  |
| JS1069 | <i>ΔphoPQ::Cm attλ::pDX1::hila'-lacZ</i>                                  |                                 |                                  |
| JS1070 | <i>Δfnr2::Tet ΔphoPQ::Cm attλ::pDX1::hila'-lacZ</i>                       |                                 |                                  |
| JS1071 | <i>Δhha1::Cm</i>  | 528131-528349                   |                                  |
| JS1072 | <i>Δhha1::Cm attλ::pDX1::hila'-lacZ</i>                                   |                                 |                                  |
| JS1073 | <i>Δhha1::Cm ΔhilD138::Kn attλ::pDX1::hila'-lacZ</i>                      |                                 |                                  |
| JS1074 | <i>Δhha1::Cm tetRA-rtxA attλ::pDX1::hila'-lacZ</i>                        |                                 |                                  |
| JS1075 | <i>Δhha1::Cm ΔhilD138::Kn tetRA-rtxA attλ::pDX1::hila'-lacZ</i>           |                                 |                                  |
| JS1076 | $\Phi(hilD'-lac^+)$ 114 <i>Δhha1::Cm</i>                                  |                                 |                                  |
| JS1077 | $\Phi(hilD'-lacZ)hyb139$ <i>Δhha1::Cm</i>                                 |                                 |                                  |

| Name   | Genotype <sup>a</sup>   | Deletion endpoints <sup>b</sup> | Source or reference <sup>c</sup> |
|--------|---|---------------------------------|----------------------------------|
| JS1078 | <i>fis-3::Cm</i>  |                                 | (72), listed as JG1160)          |
| JS1079 | <i>fis-3::Cm attλ::pDX1::hila'-lacZ</i>                                       |                                 |                                  |
| JS1080 | <i>fis-3::Cm Δhild138::Kn attλ::pDX1::hila'-lacZ</i>                          |                                 |                                  |
| JS1081 | <i>fis-3::Cm tetRA-rtsA attλ::pDX1::hila'-lacZ</i>                            |                                 |                                  |
| JS1082 | <i>fis-3::Cm Δhild138::Kn tetRA-rtsA attλ::pDX1::hila'-lacZ</i>               |                                 |                                  |
| JS1083 | Φ( <i>hild'-lac<sup>+</sup></i> )114 <i>fis-3::Cm</i>                         |                                 |                                  |
| JS1084 | Φ( <i>hild'-lacZ</i> ) <i>hyb139 fis-3::Cm</i>                                |                                 |                                  |
| JS1085 | <i>ΔhupA121::Cm</i>   | 4386709-<br>4386981             |                                  |
| JS1086 | <i>ΔhupB122::Cm</i>   | 508105-508378                   |                                  |
| JS1087 | <i>ΔhupA121:: ΔhupB122::Cm attλ::pDX1::hila'-lacZ</i>                         |                                 |                                  |
| JS1088 | <i>ΔhupA121:: ΔhupB122::Cm Δhild138::Kn attλ::pDX1::hila'-lacZ</i>            |                                 |                                  |
| JS1089 | <i>ΔhupA121:: ΔhupB122::Cm tetRA-rtsA attλ::pDX1::hila'-lacZ</i>              |                                 |                                  |
| JS1090 | <i>ΔhupA121:: ΔhupB122::Cm Δhild138::Kn tetRA-rtsA attλ::pDX1::hila'-lacZ</i> |                                 |                                  |
| JS1091 | Φ( <i>hild'-lac<sup>+</sup></i> )114 <i>ΔhupA121:: ΔhupB122::Cm</i>           |                                 |                                  |
| JS1092 | Φ( <i>hild'-lacZ</i> ) <i>hyb139 ΔhupA121:: ΔhupB122::Cm</i>                  |                                 |                                  |
| JS1093 | <i>ΔrfaH4531::Cm</i>  | 4182923-<br>4183411             |                                  |
| JS1094 | <i>ΔrfaH4531::Cm attλ::pDX1::hila'-lacZ</i>                                   |                                 |                                  |
| JS1095 | <i>ΔrfaH4531::Cm Δhild138::Kn attλ::pDX1::hila'-lacZ</i>                      |                                 |                                  |
| JS1096 | <i>ΔrfaH4531::Cm tetRA-rtsA attλ::pDX1::hila'-lacZ</i>                        |                                 |                                  |
| JS1097 | <i>ΔrfaH4531::Cm Δhild138::Kn tetRA-rtsA attλ::pDX1::hila'-lacZ</i>           |                                 |                                  |
| JS1098 | Φ( <i>hild'-lac<sup>+</sup></i> )114 <i>ΔrfaH4531::Cm</i>                     |                                 |                                  |
| JS1099 | Φ( <i>hild'-lacZ</i> ) <i>hyb139 ΔrfaH4531::Cm</i>                            |                                 |                                  |
| JS1100 | <i>ΔrelA81::Kn</i>  | 3102853-<br>3105080             |                                  |
| JS1101 | <i>ΔrelA81 ΔspoT292::Cm attλ::pDX1::hila'-lacZ</i>                            |                                 |                                  |
| JS1102 | <i>ΔrelA81 ΔspoT292::Cm Δhild138::Kn attλ::pDX1::hila'-lacZ</i>               |                                 |                                  |
| JS1103 | <i>ΔrelA81 ΔspoT292::Cm tetRA-rtsA attλ::pDX1::hila'-lacZ</i>                 |                                 |                                  |
| JS1104 | <i>ΔrelA81 ΔspoT292::Cm Δhild138::Kn tetRA-rtsA attλ::pDX1::hila'-lacZ</i>    |                                 |                                  |
| JS1105 | Φ( <i>hild'-lac<sup>+</sup></i> )114 <i>ΔrelA81 ΔspoT292::Cm</i>              |                                 |                                  |
| JS1106 | Φ( <i>hild'-lacZ</i> ) <i>hyb139 ΔrelA81 ΔspoT292::Cm</i>                     |                                 |                                  |
| JS1107 | <i>Δfur41::Cm attλ::pDX1::hila'-lacZ</i>                                      |                                 |                                  |
| JS1108 | <i>Δfur41::Cm Δhild138::Kn attλ::pDX1::hila'-lacZ</i>                         |                                 |                                  |
| JS1109 | <i>Δfur41::Cm tetRA-rtsA attλ::pDX1::hila'-lacZ</i>                           |                                 |                                  |

| Name   | Genotype <sup>a</sup>   | Deletion endpoints <sup>b</sup> | Source or reference <sup>c</sup> |
|--------|---|---------------------------------|----------------------------------|
| JS1110 | <i>Δfur41::Cm ΔhilD138::Kn tetRA-rtsA attλ::pDX1::hila'-lacZ</i>          |                                 |                                  |
| JS1111 | <i>Φ(hilD'-lac<sup>+</sup>)114 Δfur41::Cm</i>                             |                                 |                                  |
| JS1112 | <i>Φ(hilD'-lacZ)hyb139 Δfur41::Cm</i>                                     |                                 |                                  |
| JS1113 | <i>attλ::pDX1::hila'-lacZ hilD138</i>                                     |                                 |                                  |
| JS1114 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042</i>                                   |                                 |                                  |
| JS1115 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ</i>                       |                                 |                                  |
| JS1116 | <i>attλ::pDX1::hila'-lacZ hilD138 Δhile115::Cm</i>                        |                                 |                                  |
| JS1117 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 Δhile115::Cm</i>                      |                                 |                                  |
| JS1118 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ Δhile115::Cm</i>          |                                 |                                  |
| JS1119 | <i>attλ::pDX1::hila'-lacZ hilD138 ΔenvZ182::Cm</i>                        |                                 |                                  |
| JS1120 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 ΔenvZ182::Cm</i>                      |                                 |                                  |
| JS1121 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ ΔenvZ182::Cm</i>          |                                 |                                  |
| JS1122 | <i>attλ::pDX1::hila'-lacZ hilD138 ΔfadD21::Kn</i>                         |                                 |                                  |
| JS1123 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 ΔfadD21::Kn</i>                       |                                 |                                  |
| JS1124 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ ΔfadD21::Kn</i>           |                                 |                                  |
| JS1125 | <i>attλ::pDX1::hila'-lacZ hilD138 sirA3::Cm</i>                           |                                 |                                  |
| JS1126 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 sirA3::Cm</i>                         |                                 |                                  |
| JS1127 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ sirA3::Cm</i>             |                                 |                                  |
| JS1128 | <i>attλ::pDX1::hila'-lacZ hilD138 Δdam241::Cm</i>                         |                                 |                                  |
| JS1129 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 Δdam241::Cm</i>                       |                                 |                                  |
| JS1130 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ Δdam241::Cm</i>           |                                 |                                  |
| JS1131 | <i>attλ::pDX1::hila'-lacZ hilD138 Δ(ack-pta)4202::Cm</i>                  |                                 |                                  |
| JS1132 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 Δ(ack-pta)4202::Cm</i>                |                                 |                                  |
| JS1133 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ Δ(ack-pta)4202::Cm</i>    |                                 |                                  |
| JS1134 | <i>attλ::pDX1::hila'-lacZ hilD138 ΔyfgL611::Cm</i>                        |                                 |                                  |
| JS1135 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 ΔyfgL611::Cm</i>                      |                                 |                                  |
| JS1136 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ ΔyfgL611::Cm</i>          |                                 |                                  |
| JS1137 | <i>attλ::pDX1::hila'-lacZ hilD138 Δhha1::Cm</i>                           |                                 |                                  |
| JS1138 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 Δhha1::Cm</i>                         |                                 |                                  |
| JS1139 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ Δhha1::Cm</i>             |                                 |                                  |
| JS1140 | <i>attλ::pDX1::hila'-lacZ hilD138 fis-3::Cm</i>                           |                                 |                                  |
| JS1141 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 Δfis::Cm</i>                          |                                 |                                  |
| JS1142 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ fis-3::Cm</i>             |                                 |                                  |
| JS1143 | <i>attλ::pDX1::hila'-lacZ hilD138 ΔhupA121 ΔhupB122::Cm</i>               |                                 |                                  |
| JS1144 | <i>attλ::pDX1::hila'-lacZ ΔfliZ8042 ΔhupA121 ΔhupB122::Cm</i>             |                                 |                                  |
| JS1145 | <i>attλ::pDX1::hila'-lacZ ΔflhDC8045 tetRA-fliZ ΔhupA121 ΔhupB122::Cm</i> |                                 |                                  |
| JS1146 | <i>attλ::pDX1::hila'-lacZ hilD138 ΔrfaH4531::Cm</i>                       |                                 |                                  |

| Name              | Genotype <sup>a</sup>  | Deletion endpoints <sup>b</sup> | Source or reference <sup>c</sup> |
|-------------------|--|---------------------------------|----------------------------------|
| JS1147            | <i>attλ::pDX1::hilA'-lacZ ΔfliZ8042 ΔrfaH4531::Cm</i>                        |                                 |                                  |
| JS1148            | <i>attλ::pDX1::hilA'-lacZ ΔflhDC8045 tetRA-fliZ ΔrfaH4531::Cm</i>            |                                 |                                  |
| JS1149            | <i>attλ::pDX1::hilA'-lacZ hild138 ΔrelA81::Kn ΔspoT292::Cm</i>               |                                 |                                  |
| JS1150            | <i>attλ::pDX1::hilA'-lacZ ΔfliZ8042 ΔrelA81::Kn ΔspoT292::Cm</i>             |                                 |                                  |
| JS1151            | <i>attλ::pDX1::hilA'-lacZ ΔflhDC8045 tetRA-fliZ ΔrelA81::Kn ΔspoT292::Cm</i> |                                 |                                  |
| JS1152            | <i>attλ::pDX1::hilA'-lacZ hild138 Δfur41::Cm</i>                             |                                 |                                  |
| JS1153            | <i>attλ::pDX1::hilA'-lacZ ΔfliZ8042 Δfur41::Cm</i>                           |                                 |                                  |
| JS1154            | <i>attλ::pDX1::hilA'-lacZ ΔflhDC8045 tetRA-fliZ Δfur41::Cm</i>               |                                 |                                  |
| JS1155            | <i>ΔflhDC8045::Cm</i>  | 2022064-<br>2021175             |                                  |
| JS1156            | <i>attλ::pDX1::hilA'-lacZ ΔflhDC8045::Cm</i>                                 |                                 |                                  |
| JS1157            | <i>attλ::pDX1::hilA'-lacZ hild138 ΔflhDC8045::Cm</i>                         |                                 |                                  |
| JS1158            | <i>attλ::pDX1::hilA'-lacZ ΔfliZ8042 ΔflhDC8045::Cm</i>                       |                                 |                                  |
| JS1159            | <i>ΔtdcA51::Cm</i>   | 3412473-<br>3413411             |                                  |
| JS1160            | <i>attλ::pDX1::hilA'-lacZ ΔtdcA51::Cm</i>                                    |                                 |                                  |
| JS1161            | <i>attλ::pDX1::hilA'-lacZ hild138 ΔtdcA51::Cm</i>                            |                                 |                                  |
| JS1162            | <i>attλ::pDX1::hilA'-lacZ ΔfliZ8042 ΔtdcA51::Cm</i>                          |                                 |                                  |
| JS1163            | <i>attλ::pDX1::hilA'-lacZ ΔflhDC8045 tetRA-fliZ ΔtdcA51::Cm</i>              |                                 |                                  |
| Plasmids          | Relevant Characteristics   | Cloned End Points               |                                  |
| pKD46             | <i>bla</i> P <sub>BAD</sub> <i>gam bet exo</i> pSC101 oriTS                  |                                 | (24)                             |
| pCP20             | <i>bla cat cI857 λP<sub>R</sub> flp</i> pSC101 oriTS                         |                                 | (18)                             |
| pKD3              | <i>bla</i> FRT <i>cat</i> FRT PS1 PS2 oriR6K                                 |                                 | (24)                             |
| pKD4              | <i>bla</i> FRT <i>aph</i> FRT PS1 PS2 oriR6K                                 |                                 | (24)                             |
| pWKS30            | pSC101 ori, Ap <sup>f</sup>  |                                 | (106)                            |
| pRfaH<br>(pKG115) | pWKS30:: <i>rfaH</i> <sup>+</sup>  | 4182924-<br>4183440             |                                  |

<sup>a</sup> All strains are isogenic derivatives of ATCC 14028.

<sup>b</sup> The numbers indicate the base pairs that are deleted (strains) or cloned (plasmids) (inclusive) as defined in the *S. enterica* serovar Typhimurium LT2 genome sequence in the National Center for Biotechnology Information database.

<sup>c</sup> This study unless specified otherwise.

<sup>d</sup> ATCC, American Type Culture Collection.

**TABLE S3 Transcriptomic datasets that reveal co-regulation of SPI 1 and flagellar genes in *Salmonella Typhimurium***

| Global response to a regulatory system or specific environmental stimuli | Reference  | Regulates <i>hilA</i> through <i>FliZ</i> ? <sup>a</sup> | Class of SPI1 regulator |
|--|------------|--|-------------------------|
| Macrophage   | (33)       | ND   |                         |
| CsrA <sup>b</sup>  | (51)       | no   | II                      |
| Antimicrobial peptides   | (5)        | ND   |                         |
| Bile   | (81)       | no   |                         |
| Swarming   | (104; 105) | ND   |                         |
| Fis  | (48)       | no   | IV                      |
| RfaH   | (67)       | no   | IV                      |
| YfgL   | (37)       | no   | II                      |
| Fnr  | (39)       | no   | III                     |
| Hydrogen peroxide  | (40)       | ND   |                         |

<sup>a</sup> No indicates that the factor regulates *hilA* in a *fliZ* null background and when *FliZ* is ectopically expressed. ND - Not Determined

<sup>b</sup> Effect of the loss of *SirA* was tested

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