Supplemental Table 1 Oligo Primers

Primer	Sequence 5' to 3'	Description of use
CDEP1430	cacctaaggaggctttcttaatggataagagattacagc	Clone IPTG-inducible *rsiV*$^{+}$
CDEP952	ccataatcttagatggatg	Clone IPTG-inducible *rsiV*$^{+}$
CDEP1460	caccatggataagagattacagcaattaagag	Clone *gfp-rsiV*+
CDEP978	tcagttgatattaacaagcgcag	Clone *rsiV*$^{1\text{-}60}$
CDEP568	aagtcgactaaggagggtgaatttatgactataatagccgc	Clone IPTG-inducible *rasP*
CDEP569	aagcatgccttatttaaaactacatagcc	Clone IPTG-inducible *rasP*
CDEP1245	caccaagctttaaggaggatttatagatgttggcaaaaagaatcaaatgg	Clone IPTG-inducible *pdaC*
CDEP1246	aacgtacgcttaaatgtttgggccacc	Clone IPTG-inducible *pdaC*
CDEP1007	caccaacccggacgccgctcaggc	Clone *6xhis-rsiV*$^{59\text{-}285}$
CDEP209	tgtctctgtcggtgatctgg	LFH-KO *rasP::tet*
CDEP1575	caattcgccctatagtgagtcgtccgaaaataatgataaacgc	LFH-KO *rasP::tet*
CDEP1576	ccagcttttgttccctttagtgagttgtcacatggaacgatat	LFH-KO *rasP::tet*
CDEP212	gcaagctctacaacttctgc	LFH-KO *rasP::tet*
CDEP615	aaaagcttaaggaggaaggatccatgggttccgctggctccgctgctggttctggcatgagcaaaggagaagaac	Clone superfold GFP
CDEP618	aaagatctgctagcttagaattcgccagaaccagcagcggagccagcggaacctttgtagagctcatccatgc	Clone superfold GFP

Figure S1: Examples of ECF σ factor activation and a model for σ^{V} activation. Shown in green are proteases for Site-1 cleavage, in light blue the substrates, in dark blue the proteases for Site-2 cleavage, and in yellow transcription factors activated or released as a consequence of Site-2 cleavage. Red arrows point to the sites of cleavage on the substrates.

Figure S2. GFP-RsiV localizes to the membrane.

B. subtilis strains P$_{hs}$-*gfp* (JLH370) and P$_{hs}$-*gfp-rsiV* (JLH453) were grown to an OD of 0.6 before 1mM of IPTG was added. Cells were incubated for an additional 1 hour at 37^{o}C. 5 µl of culture were spotted on a 2% agarose pad and viewed using a fluorescent microscopy as previously described (1). Exposure time for images was 3 seconds. Images were taken at 12-bit, converted to JPEG and transferred to GIMP (GNU Image Manipulation Program) for crop and small contrast corrections.

1. **Tarry M, Arends SJR, Roversi P, Piette E, Sargent F, Berks BC, Weiss DS, Lea SM.** 2009. The *Escherichia coli* Cell Division Protein and Model Tat Substrate SufI (FtsP) Localizes to the Septal Ring and Has a Multicopper Oxidase-Like Structure. Journal of Molecular Biology **386:**504-519.