Table 1S. Oligonucleotide primers used in this study.

Name	Sequence"
BA00465'Kpn	TATTA <u>GGTACC</u> GGTATCCTCTGGATG
BA00463'Bam	TAGAT <u>GGATCC</u> TTTCTGAATCTAG
BA00463'Bam2	ATCCGTA <u>GGATCC</u> AACGAGATAGATC
BA00465'Nde	TTCATTTCA <u>CATATG</u> AACGTAACGAAAC
BA18775'EcoRI	TAAAC <u>GAATTC</u> CTTTCTATATTATC
BA18773'Bam	CAGTT <u>GGATCC</u> GAAATTCCATTACAC
BA18773'Bam2	GAAAG <u>GGATCC</u> TAATGTGTACTTCCT
BA18775'Nhe	TGAATG <u>GCTAGC</u> GTAAAAAGGAAGTAC
BA11875'ScaII	CTTTGT <u>CCGCGG</u> AAGAAAAGGCATAACAG
BA18773'SalI	ATATA <u>GTCGAC</u> TTATTATACTTCCATGC
BA18775'SalI	GGCGAA <u>GTCGAC</u> TGGCTGGTTTTATG
BA18773'Xho	CATCGAA <u>CTCGAG</u> ATAAAACGAATTAATG
BA24165'Kpn	AGAGA <u>GGTACC</u> GATTTGTTATGAATATG
BA24163'Bam	TCCCC <u>GGATCC</u> TGAAATGTACGTATG
BA24165'Nde	GAAGTAAAAC <u>CATATG</u> GAATTAGTGAAATTGG
BAYisi5'Eco	TAATT <u>GAATTC</u> GCATCCGTAAGAAAAAAAC
BAYisi3'Bam	TACAA <u>GGATCC</u> TTTCGTTTTTAAAATAAAC
BAYisi5'Nde	CGATT <u>CATATG</u> TTTGAGCAAGCG
BAYisI3'SalI	ATCGC <u>GTCGAC</u> AAACATTGAAATCGGCTC
BAYisI3'Sal2	CCATA <u>GTCGAC</u> AGCAAAATAAATCATTTTTCAC

a) Regions underlined denote the restriction site.

## Legend to Supplementary Figures

**Figure 1S:** MALDI TOF analysis of the BAYisI protein isolated from *E. coli*. The sequence of one possible protein resulting from the frameshift repair is shown with the His-tag sequence at the N-terminal end. The predicted molecular weight of this protein is also shown.

**Figure 2S:** Phosphorelay dephosphorylation assay. Purified Spo0E-like proteins of *B. anthracis* were tested for their ability to dephosphorylate KinA ( $0.2\mu$ M) or Spo0F ( $2.5\mu$ M). BA1877, BA0046, BA2416 and BAYisI were added at  $5\mu$ M final concentration. The reactions were initiated by the addition of ATP. Aliquots were withdrawn at the indicated times. The position of the phosphorylated KinA and Spo0F proteins is indicated by the arrows. The asterisk denotes a dimer form of Spo0F.

**Figure 3S:** Quantitation of residual phosphorylated Spo0A in the phosphorelay reactions in the presence of the *B. anthracis* Spo0E-like proteins. The 0' and 5' time points of the reaction shown in Figure 3 were quantitated using the ImageQuant software (Molecular Dynamics/Amersham). The phosphorylation level was measured in pixel and expressed as a percentage of the phosphorylation level of Spo0A in the corresponding time point of the control reaction carried out without the phosphatase.

## BAYisI (predicted MW 9,255.35) MGSSHHHHHHSSGLVPRGSHMFEQAIEKKREKMIYFAERYGMTSQKTVDCSQELDRLLNVIWHVHTDVHPNQTLDTHTQ



Bongiorni et al. Fig.1S





Bongiorni et al. Fig.2S



Bongiorni et al. Fig.3S