**Fig. S1**: Bacterial two hybrid analysis of the DivIVA interaction to MinJ and RacA. (A) The T25 plasmids p25-N-divIVA (full length DivIVA), pSBLH004 (DivIVAΔ11), pSBLH008 (DivIVAΔ20), pSBLH039 (DivIVAΔ21), pSBLH040 (DivIVAΔ26), and pSBLH041 (DivIVAΔ34), were co-transformed with the T18 plasmids pUT18C-divIVA, pUT18C-racA, and pUT18C-minJ in the *E. coli* strain BTH101 and aliquots of the transformation mixture were spotted onto nutrient agar plates containing ampicillin, kanamycin, IPTG and X-Gal (for details see materials and methods section). Images were taken after 40 h of incubation at 30°C. The T25 plasmids pKT25-racA and p25-N-minJ were used as self-interaction controls. (B) Reciprocal bacterial two hybrid experiment (taken from the same plate) in which the T25 plasmids p25-N-divIVA, pKT25-racA, and p25-N-minJ were co-transformed into BTH101 along with pUT18 plasmids containing the DivIVA truncation series. This experiment confirmed the reduced interaction of DivIVAΔ26 and DivIVAΔ34 with full-length DivIVA. However, T25 fusions of RacA and MinJ did not reveal any interactions with DivIVA in this orientation.
Fig. S2: Western blot after blue native PAGE to analyze oligomerization of chimeric DivIVA proteins. Strains expressing chimeric DivIVA proteins (BSN274: +Lm-144-Bs divIVA; BSN278: +Lm-130-Bs divIVA; BSN288: +Lm-104-Bs divIVA; BSN287: +Lm-83-Bs divIVA; BSN316: +Lm-71-Bs divIVA; BSN321: +Bs-57-Lm divIVA) were grown in LB broth supplemented with 0.5% xylose at 37°C and harvested in mid-logarithmic growth phase. Cell extracts were subjected to blue native PAGE, blotted onto a PVDF membrane and DivIVA proteins were immunostained using the anti-DivIVA antiserum. Strains 168 (wt), 4041 (ΔdivIVA) and strains expressing the B. subtilis (BSN51) or the L. monocytogenes divIVA gene (BSN238) were used as controls. Please note that differences in the apparent molecular weight of the different DivIVA oligomers are explained by pI differences between Bs (pI 5.03) and Lm DivIVA (pI 4.77). During electrophoresis, which was performed at pH7.5, Lm DivIVA is more negatively charged as compared to Bs DivIVA and therefore runs faster through the gel even though its monomer has the higher theoretical molecular weight. Moreover, the pI values of the C-terminal domains of both proteins are nearly identical (Bs CTD: 4.93; Lm CTD: 4.80) whereas those of the N-terminal lipid binding domains are rather different (Bs LBD: 5.27; Lm LBD: 4.72). This explains why all chimeras containing the Lm LBD reveal the same apparent oligomer molecular weight as Lm DivIVA, whereas the Bs-57-Lm oligomer runs at the same position as Bs DivIVA.
Fig. S3: A mutation in the tetramerisation domain (R131A) that causes an inactive Bs DivIVA protein. (A) Phase contrast micrographs obtained on cultures that were cultivated in LB broth containing 1 mM IPTG at 37°C during mid-logarithmic growth. Strains used were 168 (wt), 4041 (ΔdivIVA), BSN5 (+divIVA) and BSN313 (+R131A). Scale bar is 5 µm. (B) Western blot on cell extracts of the same set of strains. DivIVA was visualised using the polyclonal rabbit anti-DivIVA antiserum. (C) The divVAR131A mutant (strain BSN313) reveals a sporulation defect. Sporulation was assayed in a plate assay as described in the legend of Fig. 3B.
A. Subcellular localization of chimeric DivIVA proteins in a *B. subtilis* ∆*divIVA* background. Strains BSN294 (DivIVA<sup>Lm-71-Bs</sup>-GFP), BSN295 (DivIVA<sup>Lm-144-Bs</sup>-GFP), BSN296 (DivIVA<sup>Lm-130-Bs</sup>-GFP), BSN297 (DivIVA<sup>Lm-83-Bs</sup>-GFP), BSN298 (DivIVA<sup>Lm-104-Bs</sup>-GFP), and strain BSN372 (DivIVA<sup>Bs-57-Lm</sup>-GFP) were grown in LB supplemented with 0.5% xylose. DivIVA localization patterns were analysed by epifluorescence microscopy (right images), and for orientation, FM5-95 stained images (left panels) were taken in parallel. Scale bar is 5 µm, septal DivIVA-GFP signals are indicated by arrows. All DivIVA-GFP fusions contain the gfpA206K mutation preventing GFP dimerization. (B) Western blot to demonstrate full-length expression of chimeric DivIVA-GFP proteins. Strains expressing chimeric DivIVA-GFP fusions were grown in LB broth containing or not containing 0.5% xylose to mid-logarithmic growth phase, total cellular proteins were isolated and subjected to SDS-PAGE and subsequent Western blotting. DivIVA-GFP fusion proteins were visualized using the polyclonal anti-GFP antiserum. Strains were as in panel A, but BSN373 (expressing *Lm* DivIVA-GFP) was also included. For convenience the names of the respective DivIVA chimeras are indicated above the blot. Please note that DivIVA<sup>Bs-57-Lm</sup>-GFP shows some proteolytic degradation.

**Fig. S4:** Subcellular localization of chimeric DivIVA proteins in a *B. subtilis* ∆*divIVA* background. Strains BSN294 (DivIVA<sup>Lm-71-Bs</sup>-GFP), BSN295 (DivIVA<sup>Lm-144-Bs</sup>-GFP), BSN296 (DivIVA<sup>Lm-130-Bs</sup>-GFP), BSN297 (DivIVA<sup>Lm-83-Bs</sup>-GFP), BSN298 (DivIVA<sup>Lm-104-Bs</sup>-GFP), and strain BSN372 (DivIVA<sup>Bs-57-Lm</sup>-GFP) were grown in LB supplemented with 0.5% xylose. DivIVA localization patterns were analysed by epifluorescence microscopy (right images), and for orientation, FM5-95 stained images (left panels) were taken in parallel. Scale bar is 5 µm, septal DivIVA-GFP signals are indicated by arrows. All DivIVA-GFP fusions contain the gfpA206K mutation preventing GFP dimerization. (B) Western blot to demonstrate full-length expression of chimeric DivIVA-GFP proteins. Strains expressing chimeric DivIVA-GFP fusions were grown in LB broth containing or not containing 0.5% xylose to mid-logarithmic growth phase, total cellular proteins were isolated and subjected to SDS-PAGE and subsequent Western blotting. DivIVA-GFP fusion proteins were visualized using the polyclonal anti-GFP antiserum. Strains were as in panel A, but BSN373 (expressing *Lm* DivIVA-GFP) was also included. For convenience the names of the respective DivIVA chimeras are indicated above the blot. Please note that DivIVA<sup>Bs-57-Lm</sup>-GFP shows some proteolytic degradation.
Fig. S5: Mutations of R102 in Bs divIVA cause a div⁺ spo⁻ phenotype. (A) Phase contrast micrographs obtained on cultures that were cultivated in LB broth containing 1 mM IPTG (where necessary) at 37°C during mid-logarithmic growth. Strains used for this experiment were 168 (wt), 4041 (ΔdivIVA), BSN356 (+divIVA), BSN357 (+R102K), and BSN358 (+R102E). Scale bar is 5 µm. (B) Western blot on cell extracts of the same set of strains. DivIVA was visualised using the polyclonal rabbit anti-DivIVA antiserum. (C) Sporulation activity of the R102 mutants. Sporulation was assayed in a plate assay as described in the legend of Fig. 3. Please note that the sporulation defect of the phospho-ablative R102K mutant was less pronounced than that of the phospho-mimetic R102E allele.