



Figure S1, Related to Figure 1, 3, and 5. Protein stability during sporulation. (A-B) Immunoblot of cell extracts of sporulating *B. subtilis* using (A) anti-SpoIVA or (B) anti-SpoVID antisera, from cells harvested 3.5 h, 4.5 h and 6 h after the induction of sporulation with strains containing *spoIVA*^{WT} or *spoIVA*^{B*} or *spoIVA*^{T*} as indicated. Strains used : KR394, JB103, JPC221. (C) Immunoblot of cell extracts of sporulating *B. subtilis* cells harvested 3.5 h after induction of sporulation using anti-SpoVID antiserum with strains harboring *spoIVA*^{WT} or *spoIVA*^{B*} or *spoIVA*^{T*} and *spoVID*^{WT} or *spoVID*^{T532G} as indicated. Strains used : PY79, JB171, JB174, JB281, JB293, JB295, JB176, JB242. (D) Immunoblot of cell extracts of sporulating *B. subtilis* cells harvested 3.5 h after induction of sporulation using anti-GFP antiserum with strains harboring *spoIVA*^{WT} (top) or *spoIVA*^{B*} (bottom) and expressing *gfp-spoVID*⁵⁰¹⁻⁵⁷⁵, *gfp-spoVID*⁵¹¹⁻⁵⁷⁵, *gfp-spoVID*⁵¹²⁻⁵⁷⁵, *gfp-spoVID*⁵¹³⁻⁵⁷⁵, *gfp-spoVID*⁵¹⁴⁻⁵⁷⁵, *gfp-spoVID*⁵¹⁵⁻⁵⁷⁵, *gfp-spoVID*⁵¹⁶⁻⁵⁷⁵, or *gfp-spoVID*⁵²¹⁻⁵⁷⁵. Strains used: TD931, TD996, TD998, TD999, TD1000, TD1001, TD1002, TD997, TD933, TD961, TD979, TD980, TD981, TD982, TD983, TD962. Immunoblot using anti- σ^A (housekeeping transcription factor) antiserum was performed for each set of data as a loading control.

Table S1, Related to Figure 4: SpoVID intracellular concentration via immunoblot analysis, with previously reported intracellular concentrations of SpoIVA and Lipid II. Errors, when reported, are S.D.; n=3.

Molecule	Time after sporulation induction (h), or growth phase	Molecules per sporangium or cell	Reference
SpoIVA	4	270,000 ± 90,000	Peluso <i>et al.</i> , 2019
SpoVID	5.5	650,000 ± 250,000	This study
Lipid II	Logarithmic growth	18,000 (15,000 inner leaflet; 3,000 outer leaflet)	Piepenbreier <i>et al.</i> , 2019