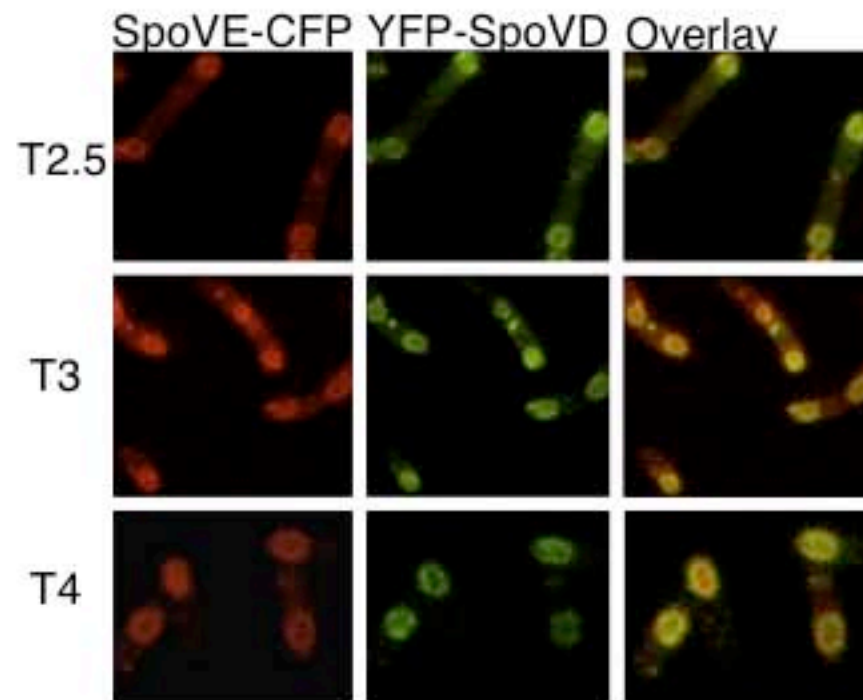


## Supp. Figure 1



**SpoVE-CFP and YFP-SpoVD co-localize to the outer forespore membrane.** Strain JDB2175 expressing SpoVE-CFP (red) and YFP-SpoVD (green) was sporulated by resuspension at 37 °C and samples taken at T2, T3 and T4 of sporulation were prepared for fluorescence microscopy.

## Supp. Figure 2



**SpoVE-SpoVD-FLAG fusion accumulates to the predicted full length size.** Strain JDB2201 expressing SpoVE-SpoVD-FLAG was sporulated by resuspension and lysates were prepared after the indicated time points post resuspension. Lysates were subjected to 10% polyacrylamide SDS-PAGE before transfer to nitrocellulose then probed with anti-FLAG (1:3000), anti-rabbit-HRP (1:25000), and detected with ECL plus.

#### Details of Strain Construction:

JDB361: PY79 transformed with pY95.2a (*PftsW-fitsW-gfp* at *amyE*) and selected with kan.

JDB389: Transformed JDB361 with pY79.2 (pDG1515 with 1kb flank upstream of *fitsW*, and 1kb flank downstream).

JDB1240: JDB646 transformed with pVK71(1) and selected for *spc*.

JDB1327: JDB1213 transformed with pKM61 and selected with *cm*.

JDB1396: PY79 transformed with pAF022 and selected with *cm*.

JDB1440: JDB1240 transformed with genomic DNA from JDB1376 and selected with *cm*.

JDB1448: PY79 transformed with pMR14 and selected with *cm*.

JDB1451: JDB1448 transformed with pCm-Er and selected with *mls*

JDB1457: JDB1440 transformed with genomic DNA from JDB1451 and selected with *mls*.

JDB1467: JDB1457 transformed with genomic DNA from JDB1213 and selected with kan.

JDB1495: JDB1440 transformed with genomic DNA prepared from PY79 which was transformed with pAF054 and selected with kan.

JDB1559: PY79 transformed with pAF078 and selected with *spc*.

JDB1636: PY79 transformed with pAF100 and selected with tet.

JDB1637: JDB1636 transformed with genomic DNA from JDB1637 and selected with *cm*.

JDB1676: JDB1134 transformed with genomic DNA from JDB1636 and selected with tet.

JDB1686: PY79 transformed with pAF104 and selected with tet.

JDB1725: PY79 transformed with pMR9 and selected with tet.

JDB1885: JDB223 transformed with genomic DNA from JDB1636 and selected with tet.

JDB1904: PY79 transformed with pMR35 and selected with *cm*.

JDB1918: JDB1752 transformed with genomic DNA from JDB1213 and selected with kan.

JDB1988: JDB1396 transformed with genomic DNA from JDB1686 and selected with cm.

JDB2026: JDB1327 transformed with genomic DNA from JDB1752 and selected with tet.

JDB2059: JDB2026 transformed with pAF211 and selected with mls.

JDB2061: JDB2026 transformed with pAF213 and selected with mls.

JDB2063: JDB2026 transformed with pAF215 and selected with mls.

JDB2064: JDB2026 transformed with pAF216 and selected with mls.

JDB2148: JDB1933 transformed with genomic DNA from JDB1213 and selected with kan.

JDB2155: JDB1933 transformed with pAF250 and selected with mls.

JDB2158: JDB2171 transformed with JDB1725 and selected with cm.

JDB2171: PY79 transformed with pMR12 and selected with cm.

JDB2200: JDB1752 transformed with pAF254 and selected with cm.

JDB2201: JDB1918 transformed with pAF254 and selected with cm.

JDB2203: JDB1918 transformed with pAF260 and selected with cm.

JDB2204: JDB1918 transformed with pAF261 and selected with cm.

JDB2255: JDB1918 transformed with pAF275 and selected with cm.

JDB2256: PY79 transformed with pAF276 and selected with cm.

JDB2266: PY79 transformed with pAF278 and selected with cm.

JDB2267: JDB1918 transformed with genomic DNA from JDB2266 and selected with cm.

JDB2269: JDB1752 transformed with genomic DNA from JDB2266 and selected with cm.

JDB2272: JDB1213 transformed with genomic DNA from JDB2204 and selected with cm.

JDB2279: JDB1213 transformed with pAF260 and selected with cm.

JDB2280: JDB1213 transformed with pAF263 and selected with cm.

JDB2321: PY79 transformed with pAF310 and selected with cm.

JDB2322: JDB1918 transformed with genomic DNA from JDB2321 and selected with cm.

JDB2362: PY79 transformed with pAF328 and selected with spc.

JDB2367: JDB1752 transformed with pAF276 and selected with cm.

JDB2439: JDB2256 with *ΔpbpB::mls* introduced using long flanking homology method. AFO701/702, AFO703/704 to amplify flanks from PY79 gDNA, and pSac-Mls as mls cassette template. *ΔpbpB::mls* confirmed by pcr and subsequent sequencing.

JDB2440: JDB2439 transformed with genomic DNA from JDB389 and selected with tet.

JDB2647: JDB2201 transformed with pAF328 and selected with spc.

JDB2661: JDB2203 transformed with pAF328 and selected for spc.

JDB2662: JDB2204 transformed with pAF328 and selected for spc.

JDB2665: JDB2269 transformed with pAF402 and selected for spc.