Supplemental Data

TABLE S1: Bacterial strains used throughout this study.

Strain	Genotype or description (species)	Source or reference
E. coli		
DH10β	Cloning host	Knight Laboratory Collection
BL21 (DE3)/pLysS	Expression host	Novagen
Bacillus		
KMS3	*gerA::spec gerB::cmΩpcm::tet gerK::erm*; PS832 (*B. subtilis*)	This study
ADL18	Wild type; PY79 (*B. subtilis*)	Driks Laboratory Collection
ADL831	Wild type; 569 (*B. cereus*)	Driks Laboratory Collection
RG1	Wild type; 34F2; $pXO1^{+}$ $pXO2^{-}$ (*B. anthracis* (Sterne))	P. Jackson
ADL2260	*bclA::kan* (*B. anthracis* (Sterne))	J. Bozue
MGM203	cotOΩpMGM3 (*B. anthracis* (Sterne))	(21)
KMS2	*exsKΔ::kan* (*B. anthracis* (Sterne))	(15)

A

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VH1a2           CTCGAGGCCGCCACCATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAA
                 L  E  A  A  T  M  E  T  G  L  R  W  L  L  L  V  A  V  L  K
VHy33           CTCGAGGCCGCCACCATGGAGACTGGGCTGCGCTGGCTTCTCCTGGTCGCTGTGCTCAAA
                 L  E  A  A  T  M  E  T  G  L  R  W  L  L  L  V  A  V  L  K
VH1a2           GGTGTCCAGTGTCAGTCGGTGAAGGAGTCCGAGGGAGGTCTCTTCAAGCCAACGGATACC
                 G  V  Q  C  Q  S  V  K  E  S  E  G  G  L  F  K  P  T  D  T
VHy33           GGTATCCAGTGTCAGCAGCTGGAGCAGTCCGGAGGAGGAGCCGGAGGAGGCCTGGTCAAG
                 G  I  Q  C  Q  Q  L  E  Q  S  G  G  G  A  G  G  G  L  V  K
VH1a2           CTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAGCAATGCAATAAGCTGGGTC
                 L  T  L  T  C  T  V  S  G  F  S  L  S  S  N  A  I  S  W  V
VHy33           CCTGGGGGATCCCTGGAACTCTGCTGCAAAGCCTCTGGATTCTCCCTCAGTAGCAGCTAC
                 P  G  G  S  L  E  L  C  C  K  A  S  G  F  S  L  S  S  S  Y
VH1a2           CGCCAGGCTCCAGGGAACGGGCTGGAATGGATCGGAGCCATTGGTAGTAGTGGTAGCGCA
                 R  Q  A  P  G  N  G  L  E  W  I  G  A  I  G  S  S  G  S  A
VHy33           TGGATATGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTTAT
                 W  I  C  W  V  R  Q  A  P  G  K  G  L  E  W  I  G  C  I  Y
VH1a2           TACTACGCGAGCTGGGCGAAAAGCCGATCCACCATCACCAGAAACACCAACCTGAACACG
                 Y  Y  A  S  W  A  K  S  R  S  T  I  T  R  N  T  N  L  N  T
VHy33           GCTGGTAGTAGTGGTAGCACTTACTATGCGAGCTGGGTGAATGGCCGATTCACTCTCTCC
                 A  G  S  S  G  S  T  Y  Y  A  S  W  V  N  G  R  F  T  L  S
VH1a2           GTGACTCTGAAAATGACCAGTCTGACAGCCGCGG
                 V  T  L  K  M  T  S  L  T  A  A
VHy33           AGAGACATCGACCAGAGCACAGGTTGCCTACAACTGAACAGTCTGACAGCCGCGG
                 R  D  I  D  Q  S  T  G  C  L  Q  L  N  S  L  T  A  A
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B

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Vκ              GGTGGAGGAGGCTCAGCTGACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGCAGCT
                 G  G  G  G  S  A  D  I  V  M  T  Q  T  P  A  S  V  E  A  A
Vλ              GGTGGAGGAGGCTCACAGCCTGTGCTGACTCAGTCGCCCTCTGCATCTGCTGCCCTGGGA
                 G  G  G  G  S  Q  P  V  L  T  Q  S  P  S  A  S  A  A  L  G
Vκ              GTGGGAGGCACAGTCACCATCAAGTGCCAGGCCAGTCAGGGCATTAGTAGTTACTTAGCC
                 V  G  G  T  V  T  I  K  C  Q  A  S  Q  G  I  S  S  Y  L  A
Vλ              TCCTCGGCCAAGCTCACCTGCACTCTGAGCAGTGCTCACAAGACCTACTATATTGAATGG
                 S  S  A  K  L  T  C  T  L  S  S  A  H  K  T  Y  Y  I  E  W
Vκ              TGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCATCCACTCTG
                 W  Y  Q  Q  K  P  G  Q  P  P  K  L  L  I  Y  R  A  S  T  L
Vλ              TATCAGCAGCAGCAAGGGGAGGCCCCTCGGTACCTGATGCAGCTTAAGAGTGATGGAAGC
                 Y  Q  Q  Q  Q  G  E  A  P  R  Y  L  M  Q  L  K  S  D  G  S
Vκ              GCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGCCGCAGTTCGCTCTCAGC
                 A  S  G  V  P  S  R  F  K  G  S  G  S  G  P  Q  F  A  L  S
Vλ              TACACCAAGGGGACCGGGGTCCCTGATCGCTTCTCGGGCTCCAGCTCTGGGGCTGACCGC
                 Y  T  K  G  T  G  V  P  D  R  F  S  G  S  S  S  G  A  D  R
Vκ              ATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAGACCTATTATTATATT
                 I  S  D  L  E  C  A  D  A  A  T  Y  Y  C  Q  T  Y  Y  Y  I
Vλ              TACTTGATCATCTCCAGCGTCCAGGCTGAGGACGAAGCTGACTACATCTGTGGTGTAACT
                 Y  L  I  I  S  S  V  Q  A  E  D  E  A  D  Y  I  C  G  V  T
Vκ              AGTGGTAGTAGTTATGGTGCTTTCGGCGGAGGGACCGAGGTGGTTGTCAAAGGTGAATTC
                 S  G  S  S  Y  G  A  F  G  G  G  T  E  V  V  V  K  G  E  F
Vλ              GGTAGTAATGTTTATGTGTTCGGCGGAGGGACCCAGCTGACCGTCACAGGTGAATTC
                 G  S  N  V  Y  V  F  G  G  G  T  Q  L  T  V  T  G  E  F
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FIGURE S1. Nucleotide and amino acid sequences of V_H and V_L germline genes used for scFv-Ig constructs. *A*, *V_H1a2* and *V_Hy33* sequences. *B*, V_κ and V_λ sequences. For details of scFv-Ig construction, refer to Materials and Methods.

TABLE S2. Identity of scFv-Ig$^+$ intestinal isolates from Fig. 1.

scFv-Ig$^+$ Isolate	**Growth conditions**	**16S rRNA gene sequence identity**
1	LB, aerobic	*Bacillus pumilus*
2	Blood agar, aerobic	*Bacillus subtilis*, *Bacillus ameloliquefaciens*[1]
3	Phenylethanol, aerobic	*Bacillus pumilus*
4	Blood agar, aerobic	*Bacillus subtilis* subspecies
5	Blood agar, anaerobic	*Bacteroides uniformis*
6	Blood agar, anaerobic	*Bacteroides ovatus*

[1]Based on the 16S rRNA gene sequence of the second isolate, we were unable to distinguish between *Bacillus subtilis* and *Bacillus ameloliquefaciens*. As a result, this isolate is designated by both names throughout the manuscript.

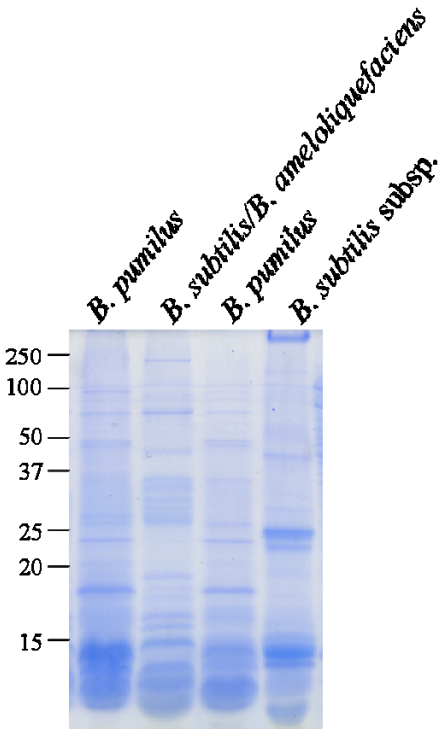

FIGURE S2. Electrophoretic analysis of extracts from intestinal *Bacillus* spores from Fig. 1*F*. To assess the efficiency of the spore protein extractions, lysates from the indicated intestinal *Bacillus* spores were analyzed by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining. While many proteins were extracted from spores, scFv-Ig bound to only a small number of proteins, demonstrating the specificity of scFv-Ig binding.

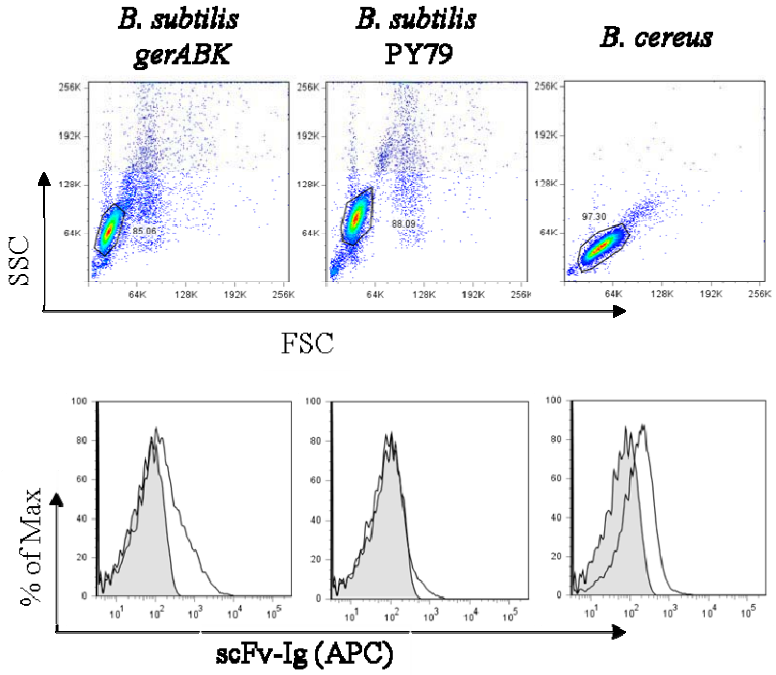

FIGURE S3. Flow cytometric analysis of *Bacillus* spores stained with scFv-Ig (related to Fig. 2). Purified spores of the indicated *Bacillus* species were stained with scFv-Ig followed by mouse anti-rabbit Fcγ and Dylight™ 649-conjugated goat Fab anti-mouse IgG. The FSC vs SSC plots are depicted in the top rows, and the scFv-Ig histograms are shown in the bottom rows. The scFv-Ig plots (unshaded histograms) were compared to staining with indirect reagents alone (shaded histograms). A limited amount of binding was observed to the surface of *B. subtilis* and *B. cereus* spores.

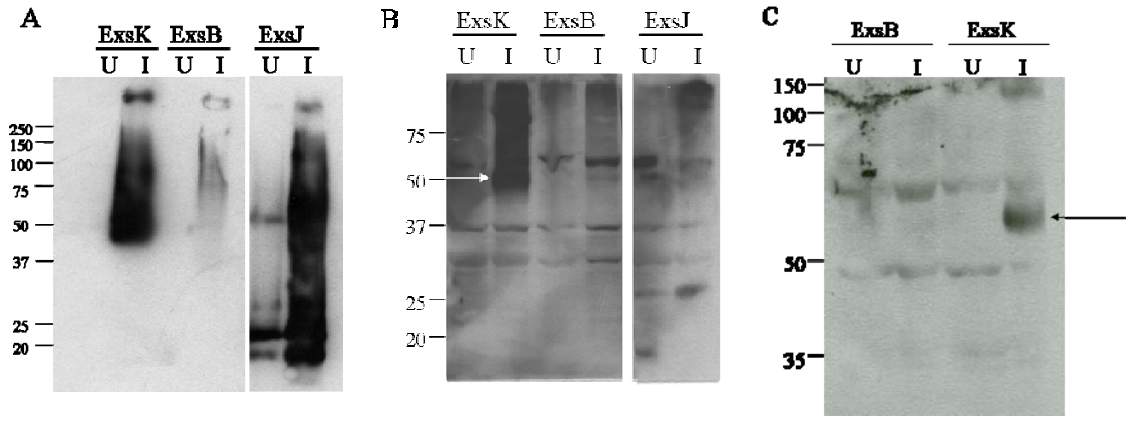

FIGURE S4. Western blot analyses of *B. anthracis* spore proteins (related to Fig. 3).

A-C, *E. coli* lysates uninduced (U) or induced (I) to produce the indicated T7-tagged *B. anthracis* protein were probed with anti-T7-HRP (A) or rabbit IgM from serum of a 6-day-old (B) or 3-week-old (C) rabbit. Arrows indicate IgM binding. These data suggest that although all four *B. anthracis* proteins were expressed, IgM bound only to ExsK.

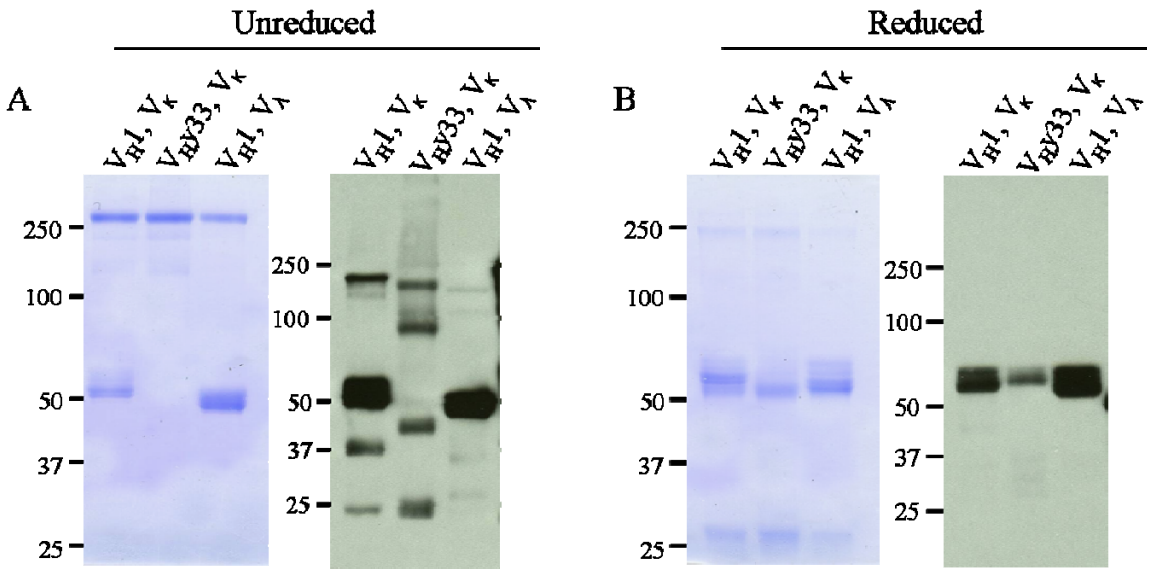

FIGURE S5. Electrophoretic analysis of scFv-Ig proteins from Fig. 4. Coomassie blue-stained SDS polyacrylamide gels (left) and western blots (right) of purified scFv-Ig proteins probed with HRP-conjugated donkey anti-rabbit IgG. Proteins were either left unreduced (*A*) or reduced (*B*) prior to gel loading to assess the purity as well as the aggregation status. *V_H1* encodes V_Ha; *V_Hy33* encodes V_Hn. These data suggest that the aggregation status of the scFv-Ig proteins does not affect binding to spores.

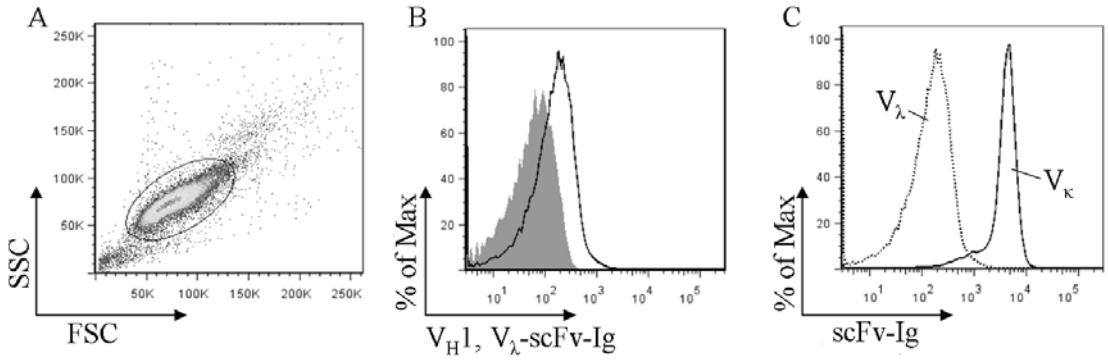

FIGURE S6. Flow cytometric analysis of *bclA* mutant *B. anthracis* spores stained with scFv-Igs from Fig. 4. *A*, FSC vs SSC dot plot spores. Gate indicates population used for staining analyses in *B* and *C*. *B*, Histograms of spores stained with V_H1, V_λ-scFv-Ig followed by mouse anti-rabbit Fcγ and FITC-conjugated goat Fab anti-mouse IgG (unshaded) or secondary antibodies alone (shaded). *C*, Histogram overlay comparing V_H1, V_κ-scFv-Ig (from Fig. 2*C*) and V_H1, V_λ-scFv-Ig staining (from part *B*). These data confirm that V_H1, V_κ-scFv-Ig binds more intensely to spores than does V_H1, V_λ-scFv-Ig.