Supplementary Information

Bacillus anthracis Poly-γ-D-Glutamate Capsule Inhibits Opsonic Phagocytosis by Impeding Complement Activation

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Supplementary Table

Table S1: List of primers used for amplifying pXO1 and pXO2 specific genes

Primers	Primer Sequence
CapA Forward Primer	TCAAGTTGTTGTCTCCACTGATACTTG
CapA Reverse Primer	GATAGTGCACTTGTGCAATATCATTTAC
AcpB Forward Primer	GTATATTGGTGTAATCGCGTTCTGTAGGG
AcpB Reverse Primer	CTGTTGAAGAAATTGCAAAAGTTACAATG
Lef Forward Primer	TTGGACTGAACATATGGCGGGCGGTCATGGT
Lef Reverse Primer	TCAGTAGGATCCGGATAGATTTATTTCTTGTTCGTTAAA

Supplementary Figure S1: Confirmation of the plasmid-encoded capsule expressing genes in *B. anthracis*

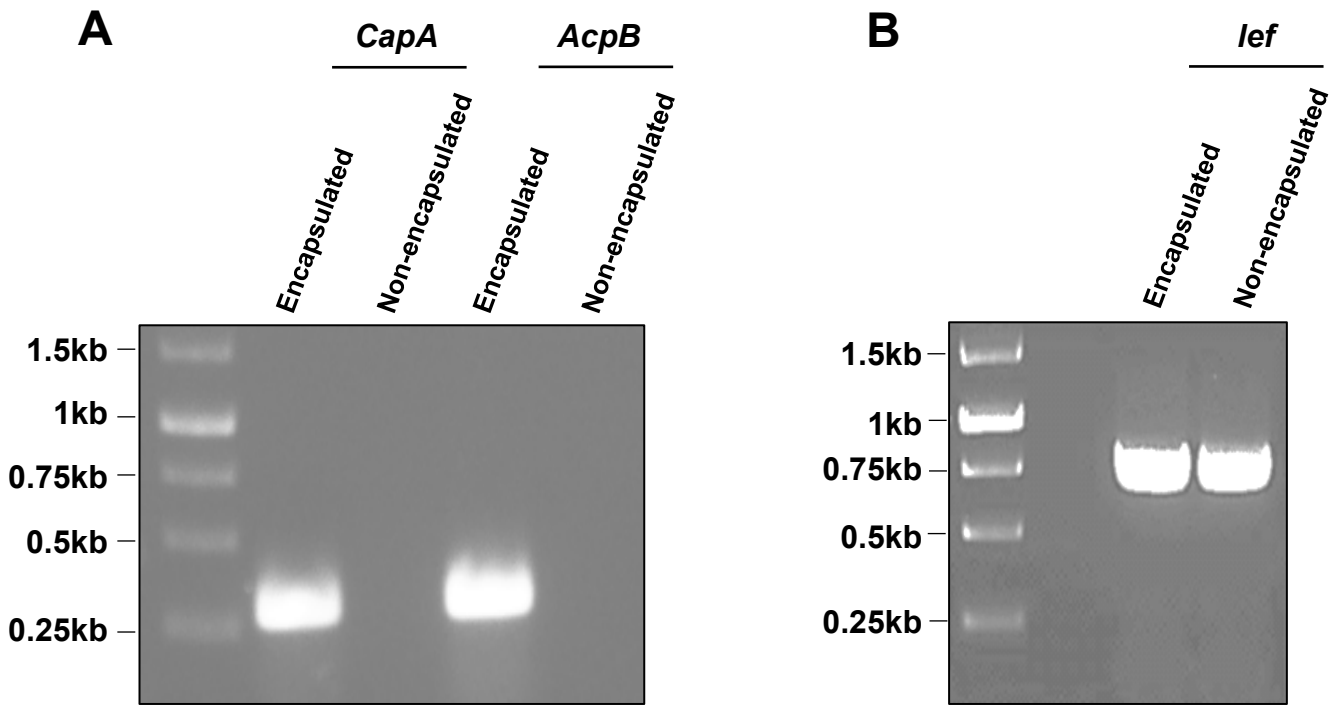

Figure S1: **Confirmation of the plasmid-encoded capsule expressing genes in *B. anthracis.*** Plasmid DNA from encapsulated and non-capsulated *B. anthracis* strains were used as templates for PCR amplification of the pXO2 and pXO1 plasmid encoded specific genes. **(A)** 300 kb amplicons for both pXO2 plasmid encoded genes, *CapA* and *AcpB.* were detected only in the encapsulated strains. **(B)** pXO1 plasmid encoded gene *lef* was amplified from both the encapsulated and non-capsulated strains.

Supplementary Figure S2: Loading controls for Immunoblot assay

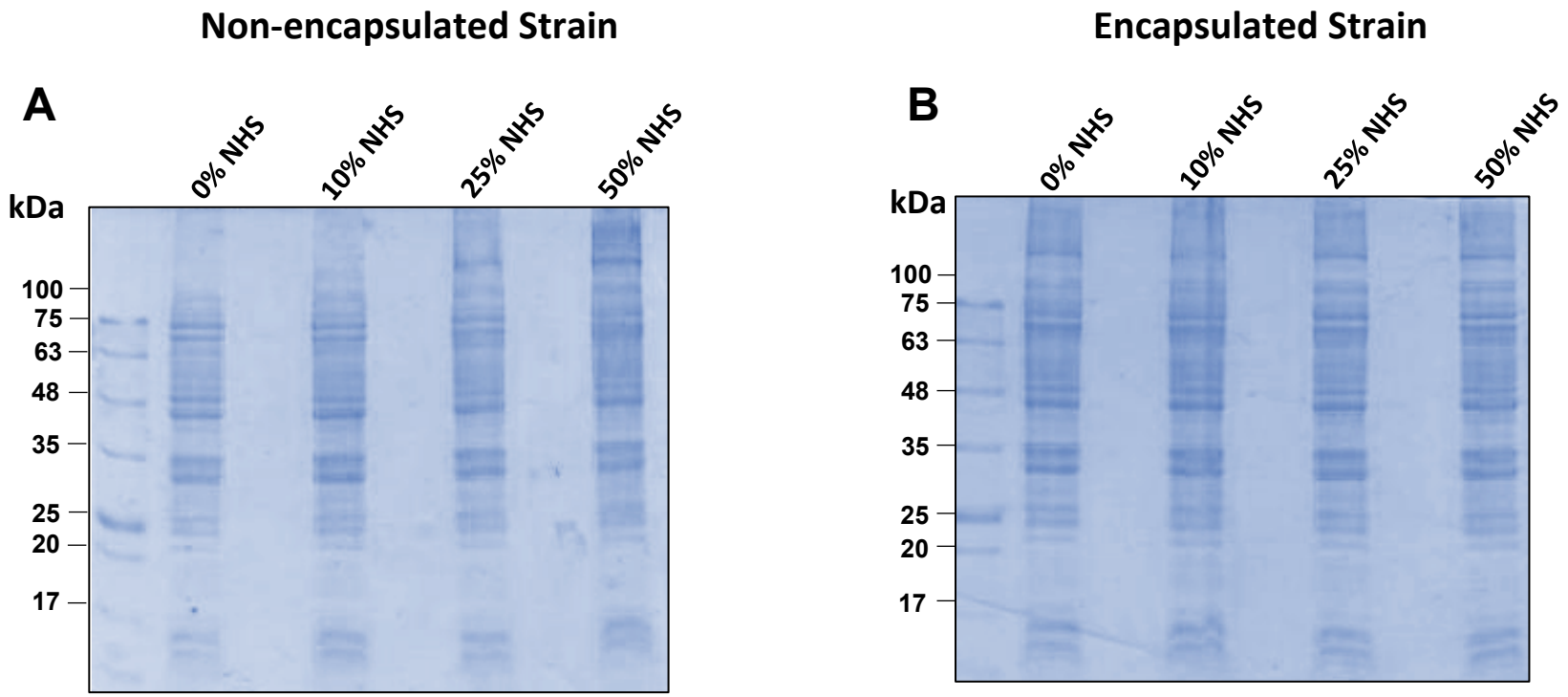

Figure S2: Loading control for immunoblot assays demonstrating serum concentration dependent binding of C3b and IgG on non-encapsulated and encapsulated strains of *B. anthracis*. Both bacterial strains were incubated with increasing concentration of normal human serum (NHS). Equal volume of bacterial lysates (representing 10^8 bacteria), as used for the immunoblot assay were added in each lane of SDS-PAGE and stained with coomassie brilliant blue G-250. SDS-PAGE profile for bacterial lysate incubated with different NHS concentration appears to be similar for both non-encapsulated strain **(A)** and encapsulated strains **(B)** of *B. anthracis*.

Supplementary Figure S3: Percentage of bacteria positive for C3b binding (Suppl. Data for Figure 2A)

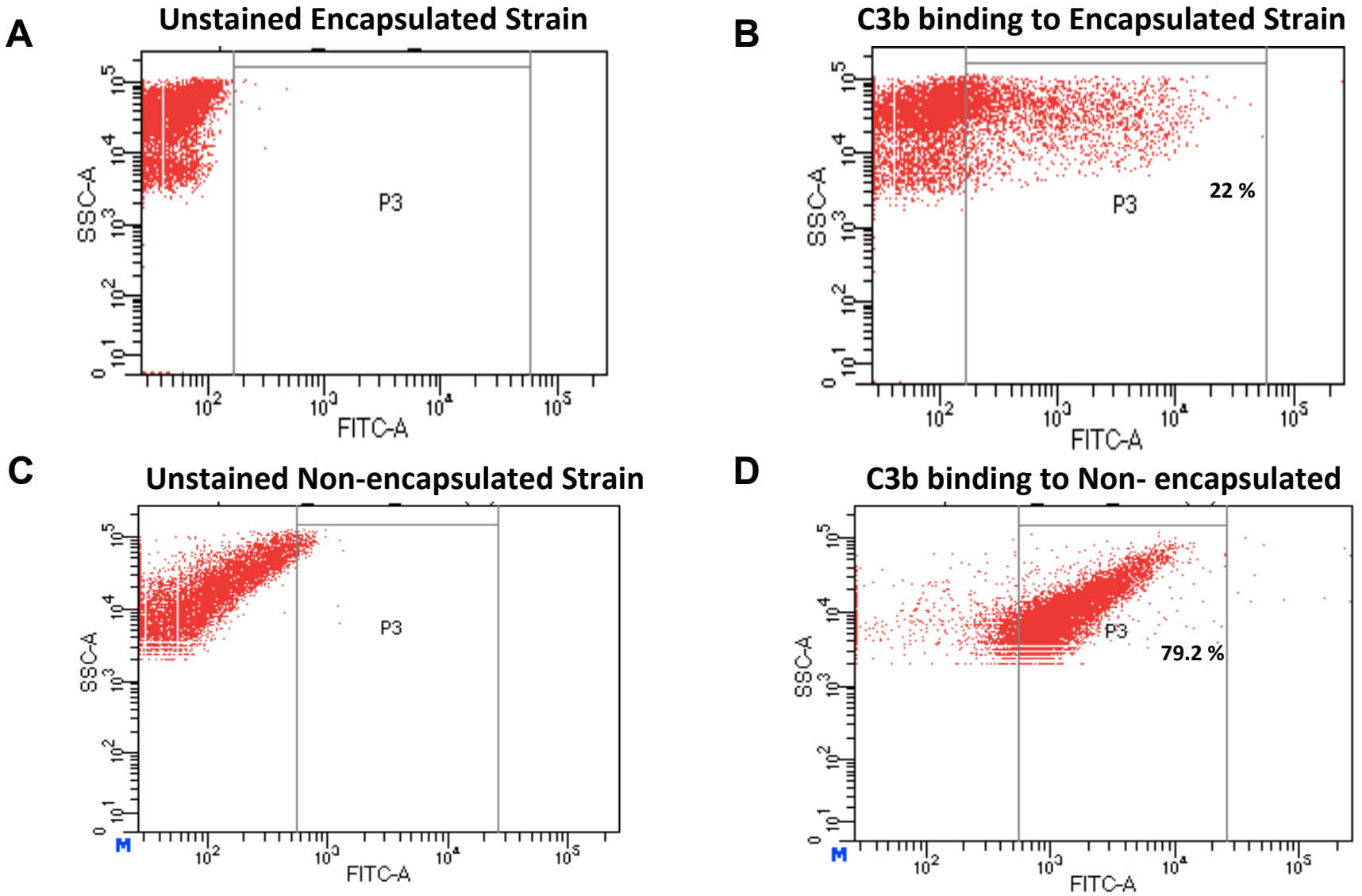

Figure S3: Flow cytometry scatter plot representing the percentage population positive for binding of C3b. Serum incubated bacterial cells probed with secondary antibodies were used as negative controls for both bacterial strains **(A & C).** A larger population of bacterial cells of the non-encapsulated *B. anthracis* strain were positive for C3b binding (79.2%) **(D)** in comparison to the encapsulated bacterial strain (22%) **(B).**

Supplementary Figure S4: Percentage of bacteria positive for CRP binding (Suppl. Data for Figure 5A)

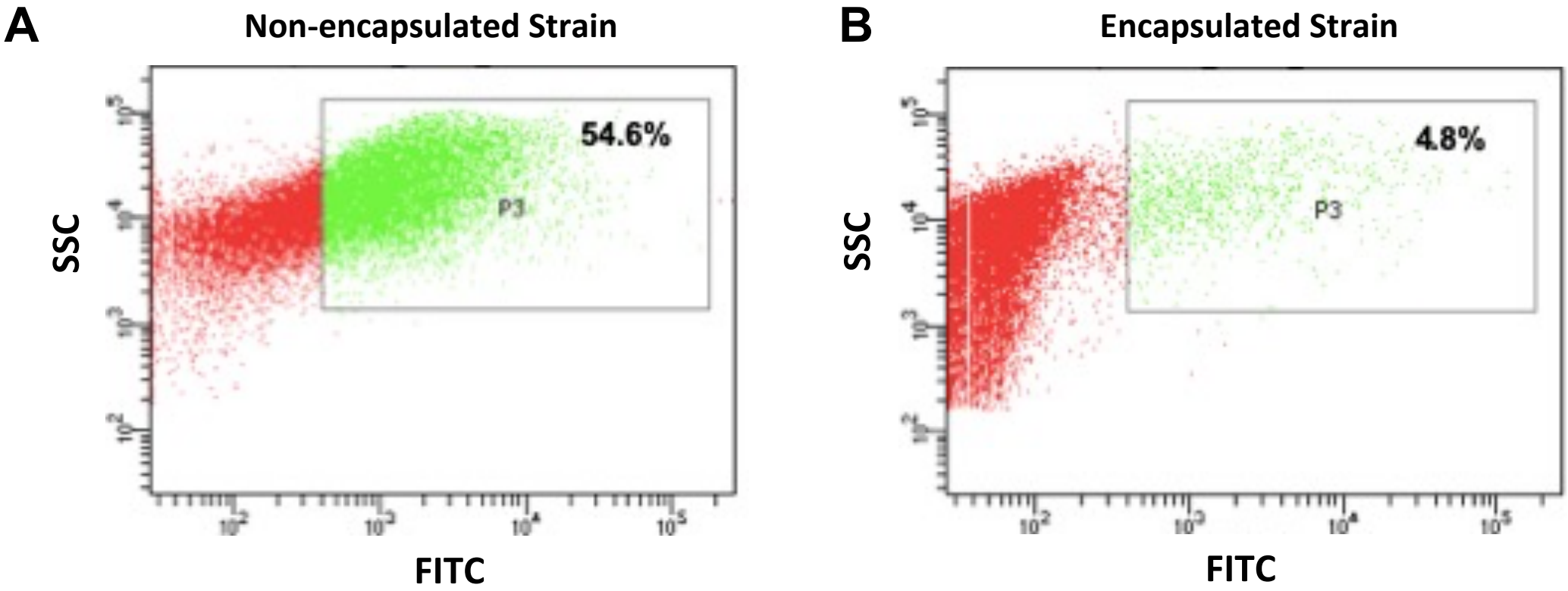

Figure S4: Flow cytometry scatter plot representing the percentage population positive for binding of CRP. Serum incubated bacterial cells probed with secondary antibodies were used as negative controls for both bacterial strains A larger population of bacterial cells of the non-encapsulated strain were positive for CRP binding (54.6%) **(A)** in comparison to the encapsulated bacterial strain (4.8%) **(B)**

Supplementary Figure S5: Percentage of positive bacteria for SAP binding (Suppl. Data for Figure 5B)

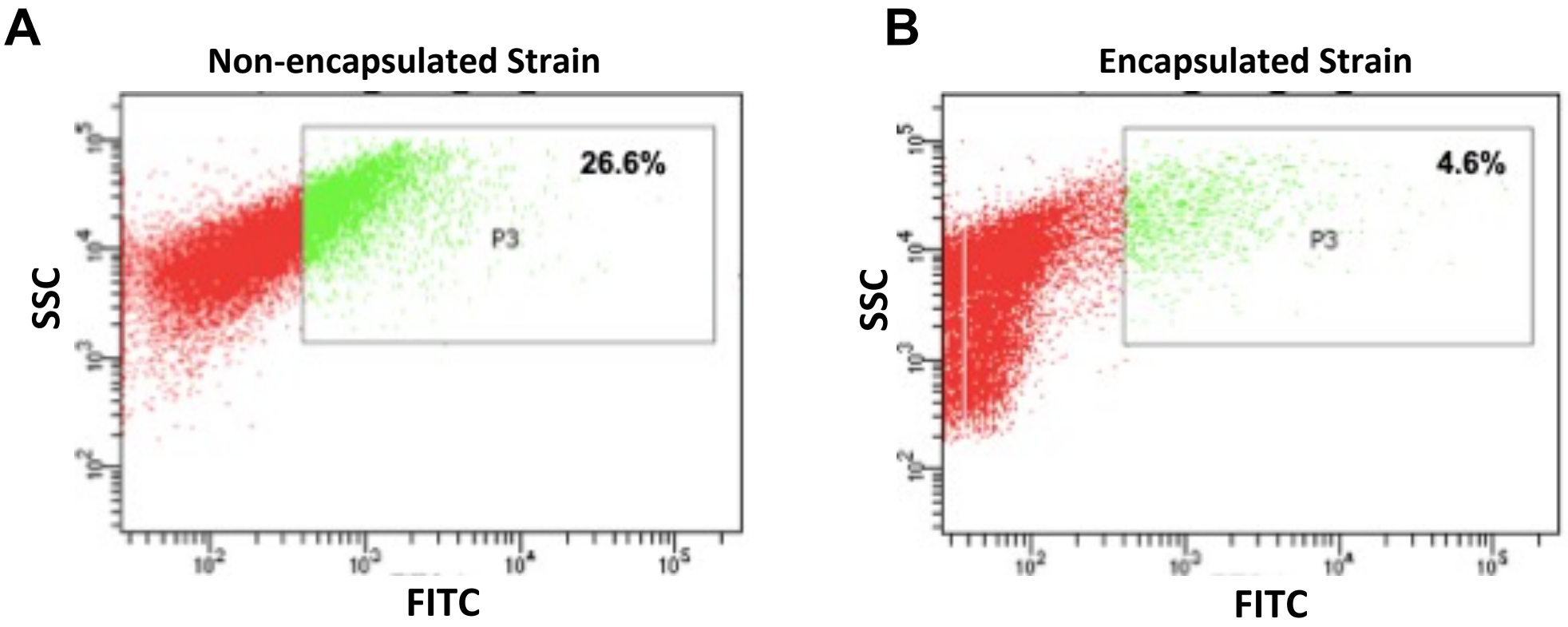

Figure S5: Flow cytometry scatter plot representing the percentage population positive for binding of SAP. Serum incubated bacterial cells probed with secondary antibodies were used as negative controls for both bacterial strains**.** A larger population of bacterial cells of the non-encapsulated strain were positive for CRP binding (26.6%) **(A)** in comparison to the encapsulated bacterial strain (4.6%) **(B).**

Supplementary Figure : Figure S6: Flow Cytometry histograms for C4BP and Factor H binding

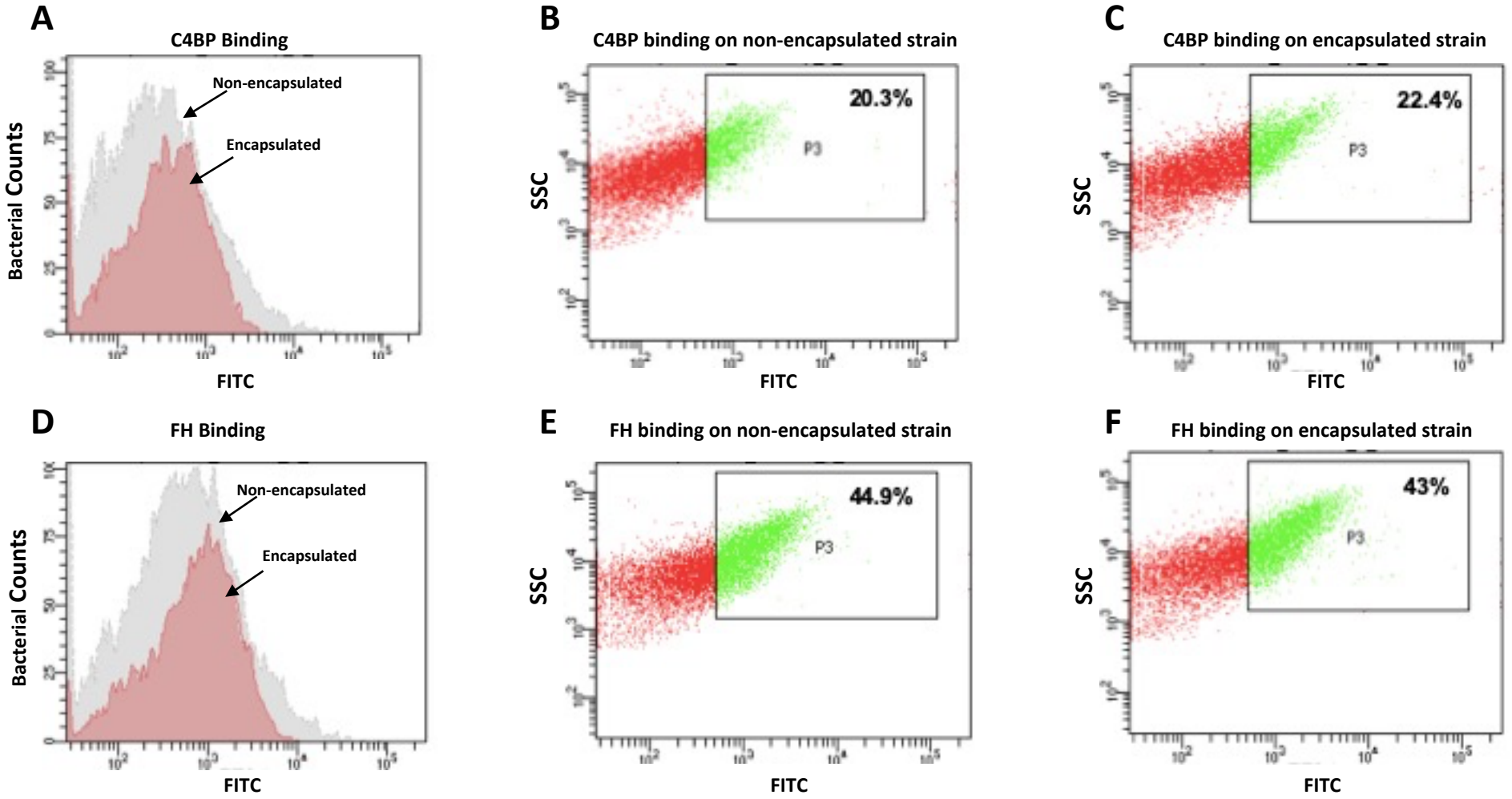

Figure S6: Flow cytometry histograms representing the binding of complement regulators C4BP **(A)** and Factor H **(D)** on encapsulated (red shade) and non-encapsulated (grey shade) *B. anthracis* strains. The binding of the complement regulators with the bacterial cells were detected by anti-human C4BP and anti-human Factor H antibodies. Percent positive population for C4BP binding with non-encapsulated **(B)** and encapsulated **(C)** *B. anthracis* strains. Percent positive population for FH binding with non-encapsulated **(B)** and encapsulated **(C)** *B. anthracis* strains. No significant difference was observed in the binding of C4BP & FH between the encapsulated and non-encapsulated strains