**Supplementary figures and tables**

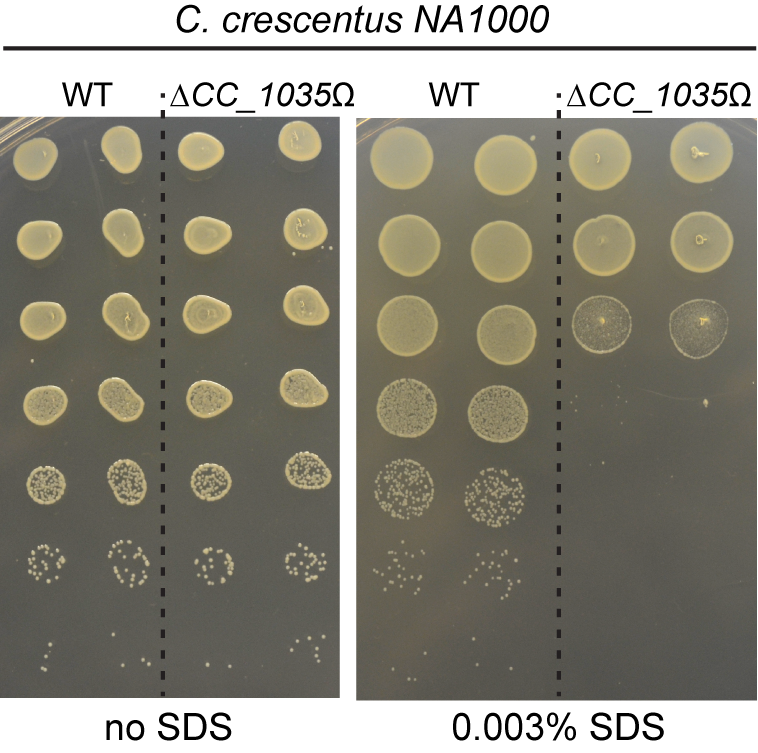
**Periplasmic protein EipA determines envelope stress resistance and virulence in *Brucella abortus***

**Julien Herrou, Jonathan W. Willett, Aretha Fiebig, Lydia M. Varieso, Daniel M. Czyż, Jason X. Cheng, Eveline Ultee, Ariane Briegel, Lance Bigelow, Gyorgy Babnigg, Youngchang Kim, and Sean Crosson**

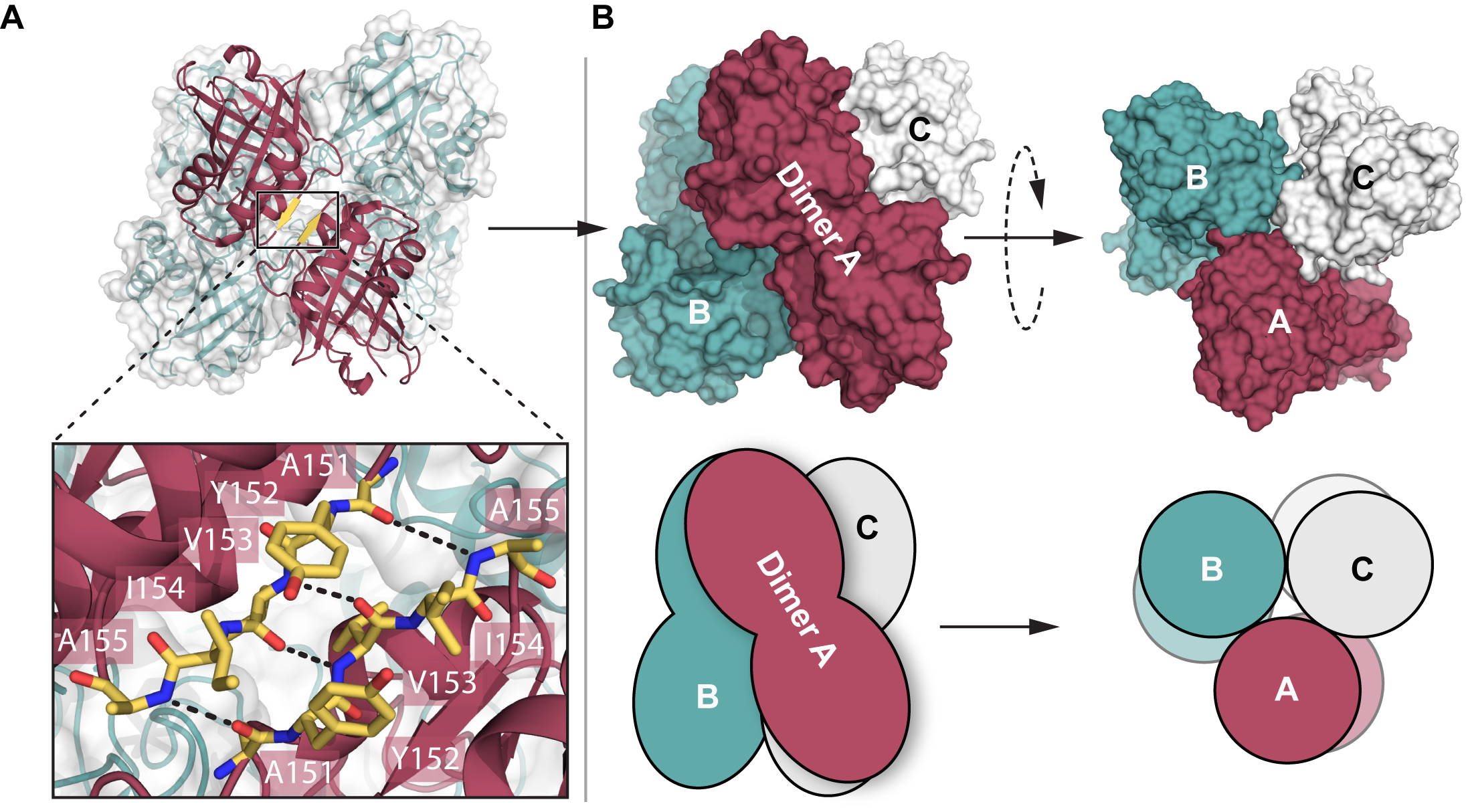
**Running Title:** Functional characterization of DUF1134 in *Alphaproteobacteria*

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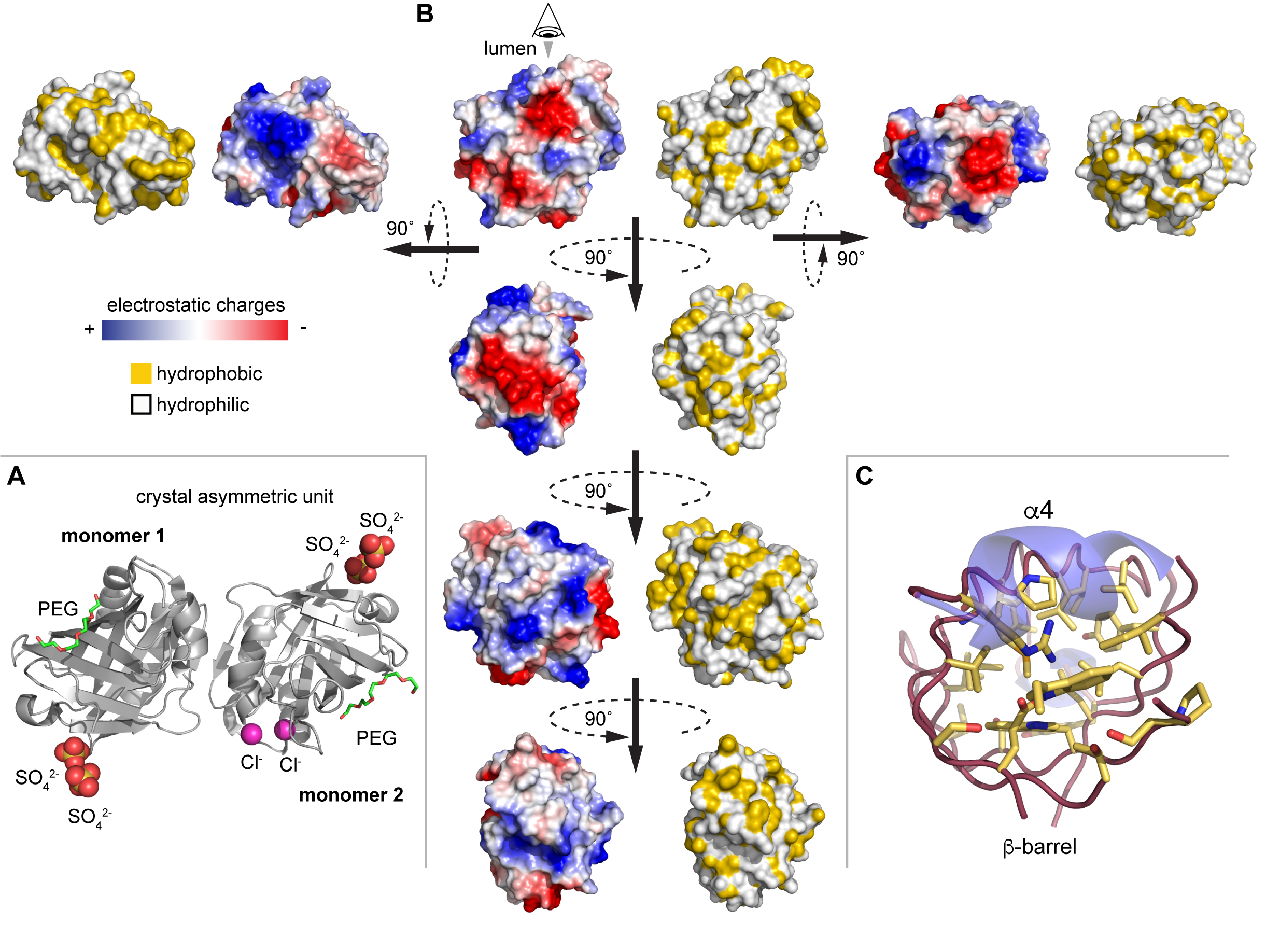
**Figure S1:** Amino acid sequence alignment of EipA (DUF1134) proteins of diverse *Alphaproteobacteria*: *B. abortus* (Bab1\_1612), *C. crescentus* (CC\_1035 / CCNA\_1087), *R. meliloti* (Smc00651), *Erythrobacter litoralis* (Eli\_00125), *Methylobacterium extorquens* (MexAM1\_META1p1722), *Bartonella quintana* (BQ09480), *Bradyrhizobium japonicum* (RN69\_37190), and *Sphingomonas melonis* (BJP26\_12640). Sequences corresponding to the peptide signal are delimited by a light-red rectangle. *B. abortus* EipA secondary structure is reported above the sequence alignment. β-strands are in turquoise and α-helices are in light brown.



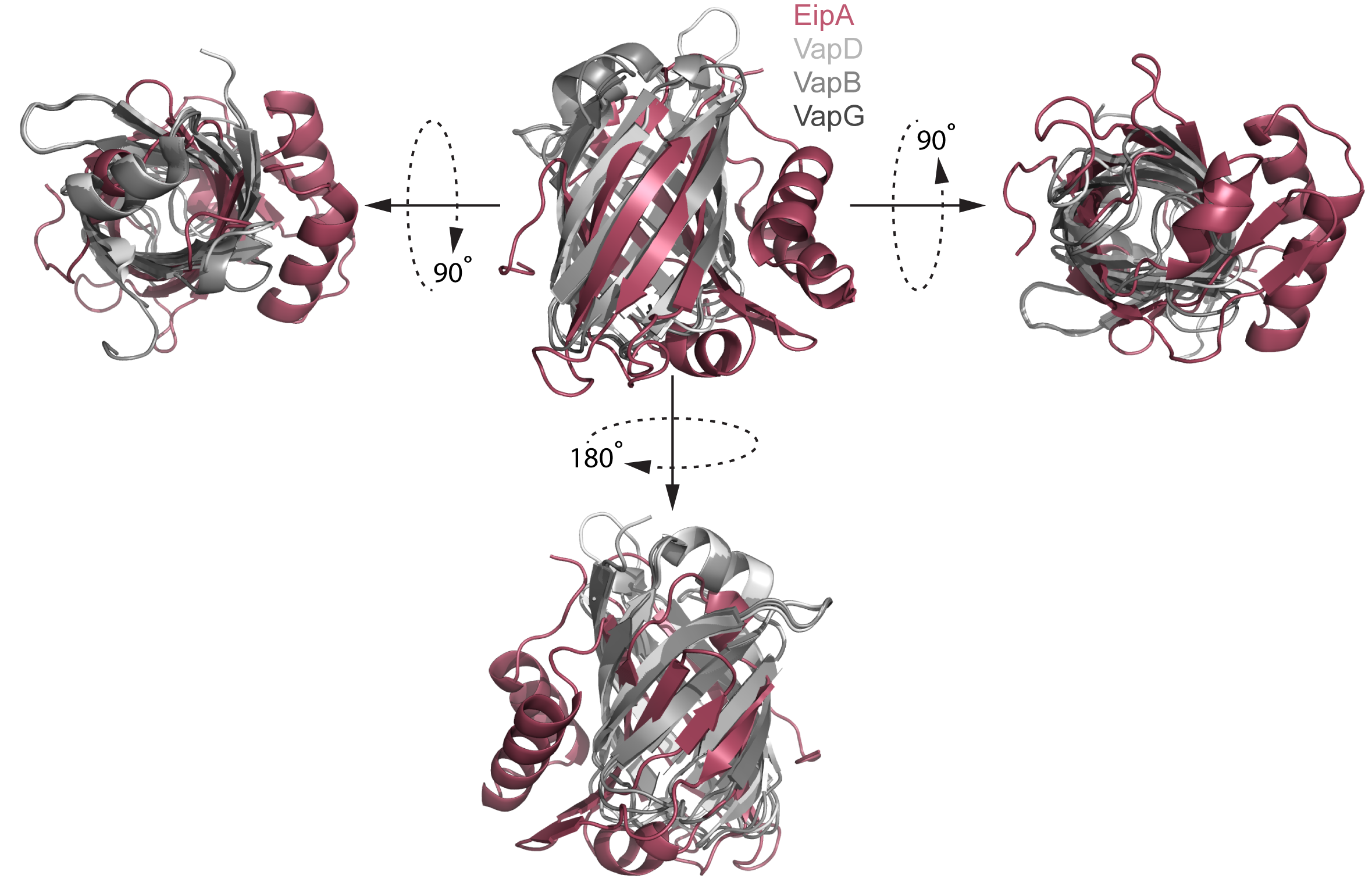
**Figure S2:** *C. crescentus* stress survival plates show that deleting gene locus *CC\_1035 / CCNA\_01087* – a homolog of *eipA* – results in sensitivity to sodium dodecyl sulfate (SDS) at high dilutions. Ten-fold serial dilutions (undiluted to 10-6, top to bottom) of log phase cultures of *C. crescentus* NA1000 strains (wild-type or ∆*CC\_1035*Ω) were spotted on plain PYE agar plates or PYE plates containing 0.003% SDS and incubated for 3 days at 30°C.



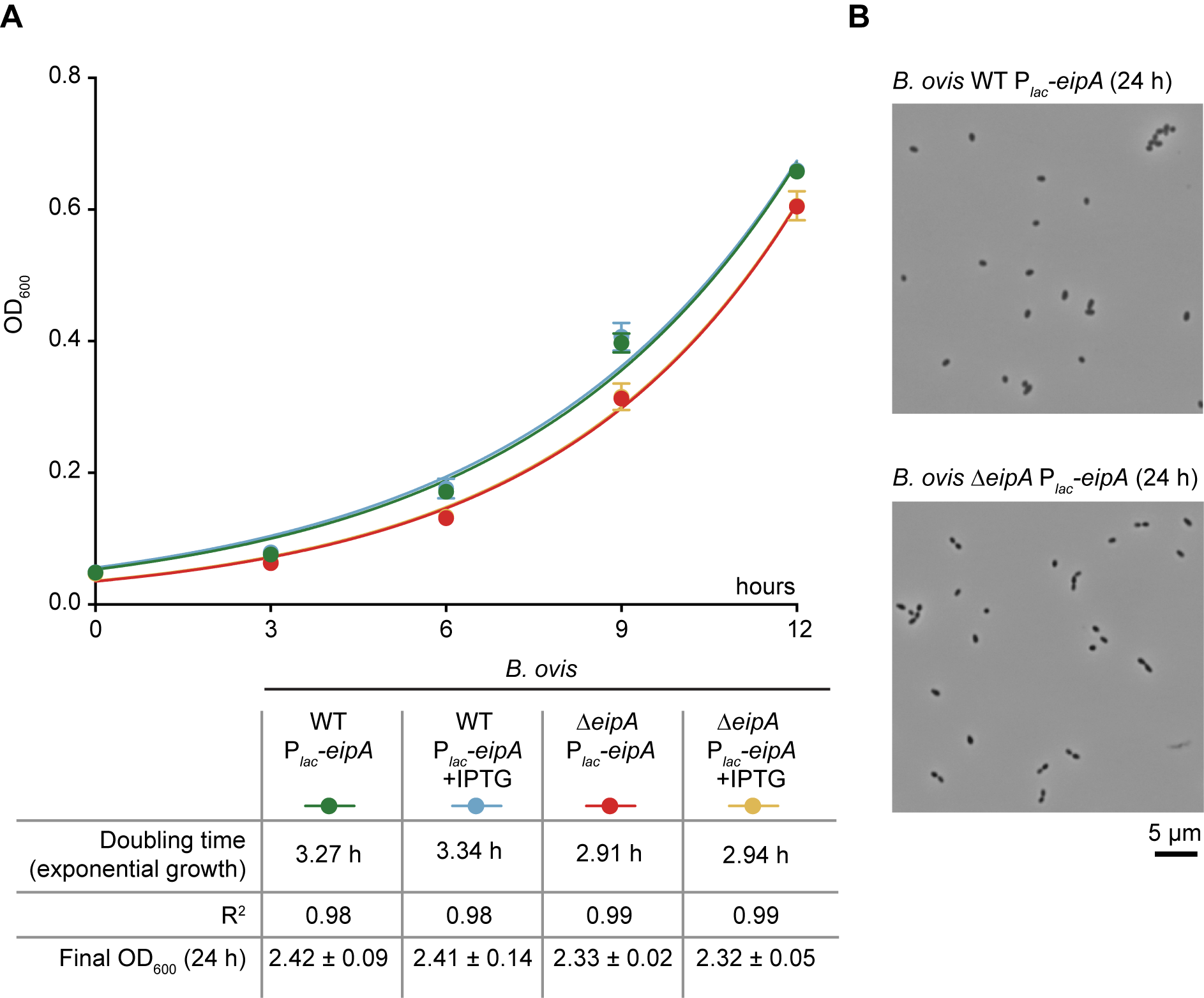
**Figure S3:** EipA interactions in the protein crystal. A) Two EipA molecules are present in the asymmetric unit of the crystal. These two proteins interact through hydrogen bonds between the β-strand 6 (in yellow) of each monomer (in maroon). Residues involved in this interaction are annotated in the inset. B) Two other dimers (in white and turquoise), present in the adjacent asymmetric units, could associate with the EipA dimer (in maroon) to form a hexameric complex of three dimers. Surface (top) and cartoon (bottom) representations of the crystal hexamer are presented. We note that at high concentrations in solution, EipA appears to be a monomer, based on size exclusion chromatography (see Figure 9).



**Figure S4:** Electrostatic and hydrophobic characteristics of EipA surface. A) In the crystal asymmetric unit, two EipA molecules are present (in grey); in addition, we modeled 4 sulfate ions (in red and yellow), 2 chloride ions (in magenta), and 2 PEG molecules (in green). B) Surface representation of EipA. Electrostatic potentials are mapped in blue for positive charges and in red for negative charges. Hydrophobicity is represented in yellow. C) Inside view of EipA β-barrel. Yellow sticks represent side chains of residues present inside the β-barrel, which are mostly hydrophobic. α-helices α3 and α4 are in transparent blue.



**Figure S5:** Structural alignment between EipA (PDB: 5UC0, in maroon), and *R. equi* VapD (PDB: 4CSB, light grey), VapB (PDB: 4CV7, grey) and VapG (PDB: 5AEO, dark grey).



**Figure S6:** Growth and morphology of the *B. ovis* *eipA* depletion strain in liquid medium. A) 12-hour liquid growth profiles of *B. ovis* wild-type and ∆*eipA* strains*.* Wild-type *B. ovis* carrying pSRK-*eipA* (P*lac*-*eipA*; IPTG-inducible) was grown in Brucella broth supplemented with (blue) or without (green) 1 mM IPTG. Similar conditions were tested with *B. ovis* ∆*eipA* carrying P*lac*-*eipA* (no IPTG: red, with IPTG: orange). The corresponding doubling times, R2 (coefficient of determination), and final ODs after 24 hours of cultivation are reported in the table below. B) Light micrographs of the corresponding strains after 24 hours of liquid growth without IPTG. The growth and morphology defects of the *B. ovis* *eipA* depletion strain cultivated on solid medium is reported in Figure 10.

**Table S1:** Summary of histopathology scoring of spleens from *B. abortus*-infected mice. Scores range from 0 to 3, and are based on masked evaluation of sectioned and stained spleen tissue. The presence of *Brucella* in tissue was confirmed by immunohistochemistry. 0 = normal (no) pathology (all naïve spleens scored 0 in all categories); 1 = mild pathology, 2 = moderate pathology, 3 = severe pathology relative to uninfected (naïve) control.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Naive** | **Wild-type** | **∆*eipA*** | **Complementation** |
| **White pulp to red pulp ratio** | Normal (1:1) | Marked decrease, (2-3) | No change, (0) | Marked decrease, (3) |
| **Average lymphoid follicles per field** | 12 | Decrease, (2) | No change, (0) | Decrease, (3) |
| **Size of follicles** | Normal | Decrease, (2) | Minimal change, (0-1) | Decrease, (2) |
| **Marginal zone depletion** | Normal (intact) | Increase, (2) | Minimal to mild increase, (0-1) | Increase, (3) |
| **Extramedullary hematopoiesis** | Minimal | Moderate to marked increase, (2-3) | Moderate increase, (2) | Marked increase, (3) |
| **Histiocytic proliferation** | Absent | Moderate to marked increase, (2-3) | Mild increase, (1) | Marked increase, (3) |
| **Granulomas** | Absent | Frequent, (3) | Rare, (1) | Frequent, (3) |
| **Brucella immunoreactivities** | Absent | Frequent, (2-3) | Very rare, (0-1) | Frequent, (3) |

**Table S2** (see Excel file Table S2)**:** List of the 1,920 growth conditions tested and the corresponding absorbance change (∆Abs630) between time=0 and 118 hours. This experiment was conducted once; concentrations of the different compounds present in this commercial screen are not available from the manufacturer. This Biolog dataset provided preliminary evidence for an envelope stress defect in the *B. abortus* ∆*eipA* strain, which was directly validated in experiments presented in Figure 5.

**Table S3:** Transposon library statistics based on analysis of Tn-sequencing data analysis using MapTnSeq.pl and DesignRandomPool.pl available at https://bitbucket.org/berkeleylab/feba. 150 bp single end read data that include the barcode and Tn-chromosome insertion junction are available through the NCBI sequence read archive at accessions SRR7943723, SRR7943724, and SRR7943771 (https://www.ncbi.nlm.nih.gov/sra).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Library** | **Estimated number of Tn strains** a | **Unique Tn strain included** b | **Unique insertion sites**c | **Total TA sites**d | **Median Tn per gene** | **Median reads per gene** |
| *B. abortus 2308* | 3.8 x 106 | 535,231 | 99,761 | 156,924 (78,462) | 86 | 1436 |
| *B. abortus 2308 ∆eipA* | 15.5 x 106 | 716,817 | 108,106 | 156,924 (78,462) | 119 | 5460 |
| *B. ovis* | 2.6 x 106 | 367,537 | 95,026 | 156,996 (78,498) | 61 | 1080 |

a Chao2 estimate of number of barcodes present in the library

b Number of unique barcodes passing mapping criteria (sequenced 5 or more times, mapped to unique site, perfect match)

c Counts hits on opposite strands as unique

d TA sites on forward and reverse complement (TA on forward only)

**Table S4:** Crystallographic data collection and refinement statistics. Statistics for the highest resolution shell are shown in parentheses.

|  |  |
| --- | --- |
|  | **EipA** |
| **Wavelength (**Å**)** | 0.97929 |
| **Resolution range (**Å**)** | 32.64 - 1.73 (1.76 - 1.73) |
| **Space group** | P63 |
| **Unit cell** | a=b=113.08 Å, c=64.31 Å, α=β=90°, γ=120° |
| **# molecules in ASU** | 2 |
| **Unique reflections** | 49076 (2420) |
| **Multiplicity** | 7.4 (7.3) |
| **Completeness (%)** | 99.7 (99.1) |
| **Mean I/sigma(I)** | 24.9 (2.2) |
| **Wilson B-factor (**Å**2)** | 23.12 |
| **R-merge** | 0.089 (0.761) |
| **cc1/2 (highest resolution shell)** | 0.799 |
| **Reflections used for R-free** | 2539 |
| **R-work** | 0.176 |
| **R-free** | 0.208 |
| **RMS(bonds)** | 0.0100 |
| **RMS(angles)** | 0.876 |
| **Ramachandran favored (%)** | 97.8 |
| **Ramachandran outliers (%)** | 0.0 |
| **Clashscore** | 8.97 |
| **Average B-factor (**Å**2)** | 25.5 |

**Table S5:** A list of the top 50 most structurally similar proteins to EipA based on a Dali protein structure comparison (http://ekhidna2.biocenter.helsinki.fi/dali/). Proteins with a high Z-score, low rmsd, and high percentage of identity were considered to be structurally-related to the query protein structure. As expected, comparison of EipA to itself (Uncharacterized protein COG5400, PDB ID: 5UC0) is the top hit.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **PDB + Chain** | **Z-score** | **rmsd** | **Length of alignment** | **# residues** | **% identity** | **Description** |  |
| 1 | 5uc0-A | 36.5 | 0 | 161 | 161 | 100 | UNCHARACTERIZED PROTEIN COG5400 | EipA |
| 2 | 5uc0-B | 33.5 | 0.3 | 159 | 159 | 100 | UNCHARACTERIZED PROTEIN COG5400 | EipA |
| 3 | 4csb-A | 7 | 3.1 | 95 | 113 | 11 | VIRULENCE ASSOCIATED PROTEIN VAPD |  |
| 4 | 5aeo-B | 6.7 | 2.9 | 93 | 110 | 13 | R. EQUI VAPG PROTEIN |  |
| 5 | 5aeo-A | 6.7 | 2.9 | 92 | 110 | 11 | R. EQUI VAPG PROTEIN |  |
| 6 | 4cv7-A | 6.4 | 2.9 | 91 | 111 | 10 | VIRULENCE ASSOCIATED PROTEIN VAPB |  |
| 7 | 3ke6-A | 4.1 | 3 | 77 | 354 | 12 | PROTEIN RV1364C/MT1410 |  |
| 8 | 2ovs-A | 3.9 | 3 | 69 | 118 | 13 | L0044 |  |
| 9 | 5l33-A | 3.6 | 2.9 | 52 | 106 | 8 | DENOVO NTF2 |  |
| 10 | 5tgn-A | 3.5 | 3.2 | 54 | 109 | 13 | UNCHARACTERIZED PROTEIN |  |
| 11 | 2b4w-A | 3.5 | 2.8 | 65 | 292 | 11 | HYPOTHETICAL PROTEIN, CONSERVED |  |
| 12 | 6f0x-P | 3.5 | 3.3 | 82 | 194 | 13 | PACHYTENE CHECKPOINT PROTEIN 2 HOMOLOG |  |
| 13 | 3ke6-B | 3.5 | 2.9 | 75 | 348 | 12 | PROTEIN RV1364C/MT1410 |  |
| 14 | 4odd-A | 3.3 | 4 | 74 | 149 | 3 | LIPOCALIN ALLERGEN |  |
| 15 | 4z6j-A | 3.3 | 3.6 | 75 | 133 | 7 | AVIDIN FAMILY |  |
| 16 | 3cnx-C | 3.2 | 3.2 | 58 | 148 | 3 | UNCHARACTERIZED PROTEIN |  |
| 17 | 6dbn-A | 3.2 | 2.8 | 51 | 281 | 8 | TYROSINE-PROTEIN KINASE JAK1 |  |
| 18 | 3d2u-E | 3.2 | 5.5 | 60 | 279 | 2 | UL18 PROTEIN |  |
| 19 | 6f92-A | 3 | 4.2 | 77 | 760 | 6 | PUTATIVE ALPHA-1,2-MANNOSIDASE |  |
| 20 | 5oqj-H | 3 | 2.9 | 68 | 136 | 4 | DNA-DIRECTED RNA POLYMERASE II SUBUNIT RPB1 |  |
| 21 | 4rul-A | 3 | 3 | 70 | 821 | 16 | DNA TOPOISOMERASE 1 |  |
| 22 | 4cgy-A | 3 | 3.2 | 70 | 619 | 6 | DNA TOPOISOMERASE 3-ALPHA |  |
| 23 | 2waq-G | 3 | 3.5 | 68 | 113 | 7 | DNA-DIRECTED RNA POLYMERASE RPO1N SUBUNIT |  |
| 24 | 4rki-A | 3 | 4.2 | 59 | 374 | 7 | DNA POLYMERASE III SUBUNIT BETA |  |
| 25 | 2jso-A | 2.9 | 3 | 63 | 88 | 10 | POLYMYXIN RESISTANCE PROTEIN PMRD |  |
| 26 | 5hnv-A | 2.9 | 2.7 | 51 | 283 | 8 | PPKA N TERMINAL |  |
| 27 | 5wce-A | 2.9 | 3.7 | 58 | 370 | 9 | DNA POLYMERASE III SUBUNIT BETA |  |
| 28 | 4ge1-A | 2.9 | 3.6 | 79 | 195 | 4 | BIOGENIC AMINE-BINDING PROTEIN |  |
| 29 | 4rlc-A | 2.9 | 4 | 76 | 135 | 8 | OUTER MEMBRANE PORIN F |  |
| 30 | 5iuq-A | 2.9 | 4.7 | 49 | 138 | 8 | GALECTIN-3 |  |
| 31 | 6d47-A | 2.8 | 3.8 | 59 | 388 | 5 | BETA SLIDING CLAMP |  |
| 32 | 2y32-C | 2.8 | 3 | 67 | 138 | 6 | BLR5658 PROTEIN |  |
| 33 | 1yem-B | 2.8 | 3.9 | 67 | 166 | 3 | CONSERVED HYPOTHETICAL PROTEIN PFU-838710-001 |  |
| 34 | 5i8u-C | 2.8 | 3.8 | 46 | 203 | 9 | ADP-RIBOSE PYROPHOSPHATASE |  |
| 35 | 6deg-B | 2.8 | 3.8 | 56 | 351 | 2 | BETA SLIDING CLAMP |  |
| 36 | 1wzn-A | 2.8 | 5.1 | 55 | 245 | 5 | SAM-DEPENDENT METHYLTRANSFERASE |  |
| 37 | 3en8-A | 2.8 | 3.2 | 53 | 128 | 9 | UNCHARACTERIZED NTF-2 LIKE PROTEIN |  |
| 38 | 4ysm-A | 2.7 | 2.6 | 48 | 475 | 10 | CALMODULIN-LIKE DOMAIN PROTEIN KINASE |  |
| 39 | 2dsb-A | 2.7 | 3.1 | 46 | 206 | 13 | ADP-SUGAR PYROPHOSPHATASE |  |
| 40 | 5izl-A | 2.7 | 4.6 | 58 | 514 | 10 | SELENOCYSTEINE-SPECIFIC ELONGATION FACTOR |  |
| 41 | 4dk0-A | 2.7 | 3.2 | 55 | 324 | 5 | PUTATIVE MACA |  |
| 42 | 6fu5-B | 2.7 | 3.6 | 49 | 286 | 8 | RECEPTOR-INTERACTING SER/THR-PROTEIN KIN |  |
| 43 | 4xrw-A | 2.7 | 3.2 | 56 | 305 | 9 | BEXL |  |
| 44 | 1z47-A | 2.7 | 4.6 | 51 | 345 | 4 | PUTATIVE ABC-TRANSPORTER ATP-BINDING PROTEIN |  |
| 45 | 4ci8-A | 2.7 | 3.6 | 58 | 640 | 7 | ECHINODERM MICROTUBULE-ASSOCIATED PROTEIN-LIKE |  |
| 46 | 3f40-A | 2.7 | 2.6 | 51 | 112 | 6 | UNCHARACTERIZED NTF2-LIKE PROTEIN |  |
| 47 | 1mm4-A | 2.7 | 3.1 | 70 | 170 | 11 | CRCA PROTEIN |  |
| 48 | 6es1-A | 2.7 | 6.9 | 64 | 420 | 5 | BOTULINUM NEUROTOXIN TYPE A |  |
| 49 | 5czo-A | 2.7 | 3.2 | 50 | 374 | 10 | CASEIN KINASE I HOMOLOG HRR25 |  |
| 50 | 3cm1-A | 2.7 | 3.5 | 75 | 137 | 4 | SSGA-LIKE SPORULATION-SPECIFIC CELL DIVISION PROT |  |

**Table S6** (see Excel file Table S6)**:** Primers used in this study.

**Table S7** (see Excel file Table S7)**:** Strains used in this study.