STRUCTURAL BIOLOGY COMMUNICATIONS

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Supporting information for article:

SpaB, an atypically adhesive basal pilin from the lactobacillar SpaCBA pilus: crystallization and X-ray diffraction analysis

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........10 ........20 ........30 ........40 ........50 ........60
MTKSFRPLVI LTFCLALLVS LATTTLQQTQ AATVPTTVDV VLHKLLFKDT LPTQQANNGT

........70 ........80 ........90 .......100 .......110 .......120
TKPDFSQADV PLNGVTFTVY DVTADFWQLV SKNGGAIEVA QTTLSQDSYQ PASSSLIAQV

.......130 .......140 .......150 .......160 .......170 .......180
VTAGQGEAYF GDLPLRQGQH AAVYLFKETA APKNIEASQN LVVVMSSNLQ HGNQSRIDLF

.......190 .......200 .......210 .......220 .......230 .......240
PKNKMVSRHT DAPKKVPKKI RQLLPQTGDT VAAWLSVLGL IIFATVLAFN IKNQKINKWE

.......250
R
```
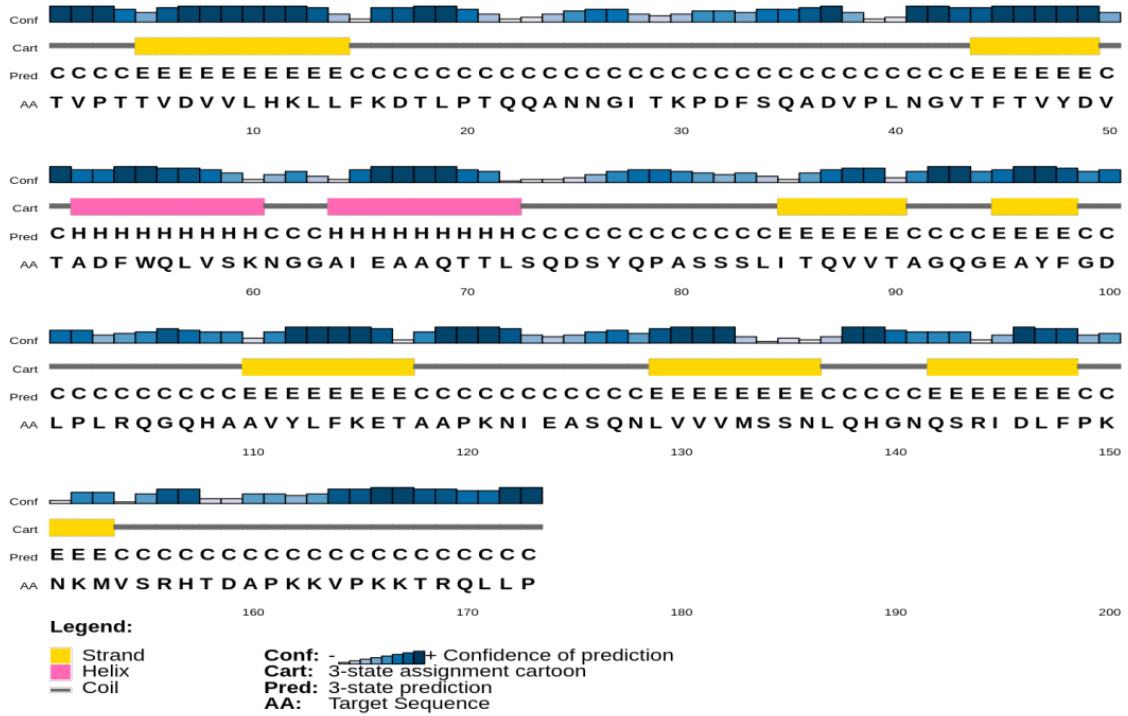
Supplementary Fig. S1: Peptide mass spectra data of hexagonal SpaB crystals.
Residue fragments identified through a search of the NCBI non-redundant protein database using the Mascot search engine (Matrix Science) are in red font and underlined.

Supplementary Fig. S2: Secondary structure prediction of *L. rhamnosus* GG SpaB by sequence analysis. PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) secondary structure predictions output of SpaB sequence is provided. Secondary structural elements (strand, helix, and coil) and their confidence levels are indicated.
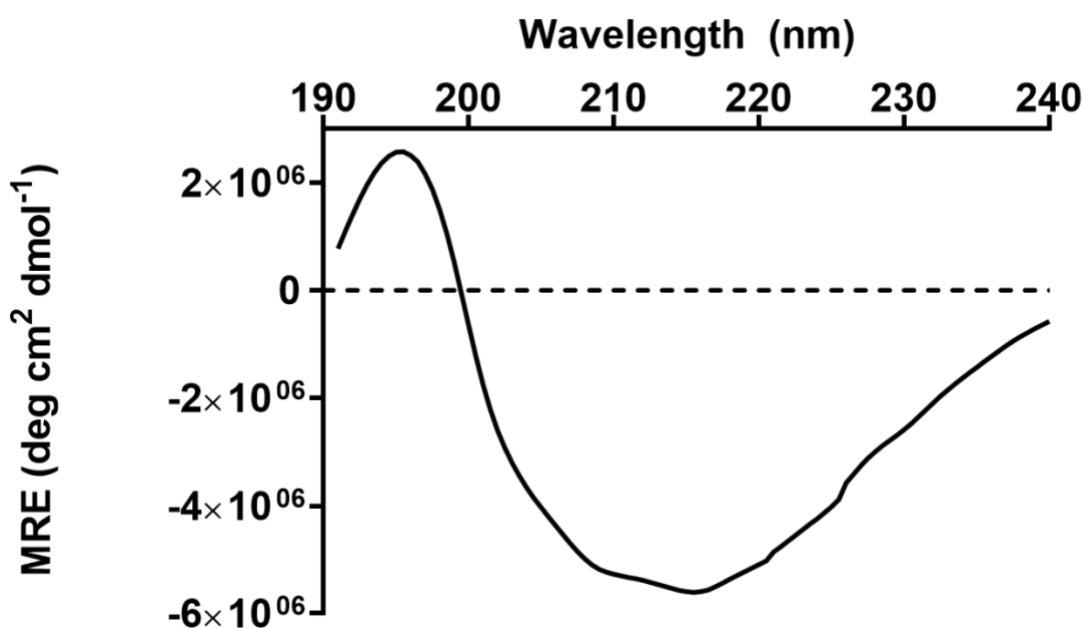
Supplementary Fig. S3: Secondary structure prediction of *L. rhamnosus* GG SpaB by circular dichroism (CD) spectrum analysis. Recombinant SpaB protein (0.2 mg ml-1 in 25 mM phosphate buffer pH 8.0 in a quartz cuvette of 0.1 cm path length) underwent CD analysis at 295 K with a Jasco J-815 CD spectrophotometer. A far-UV CD spectrum covered 190-240 nm and was recorded using a 1.0 nm band width, a data interval of 0.1 nm, and a one second signal averaging time. Plotting of the spectrum is in units of mean molar residue ellipticity minus the buffer scans. A Jasco J-810 spectro-polarimeter served to analyse the CD spectrum, which showed that the percentage composition of the secondary structural elements includes ~53% β-sheets, ~40% random coils, and ~0.5% turns.
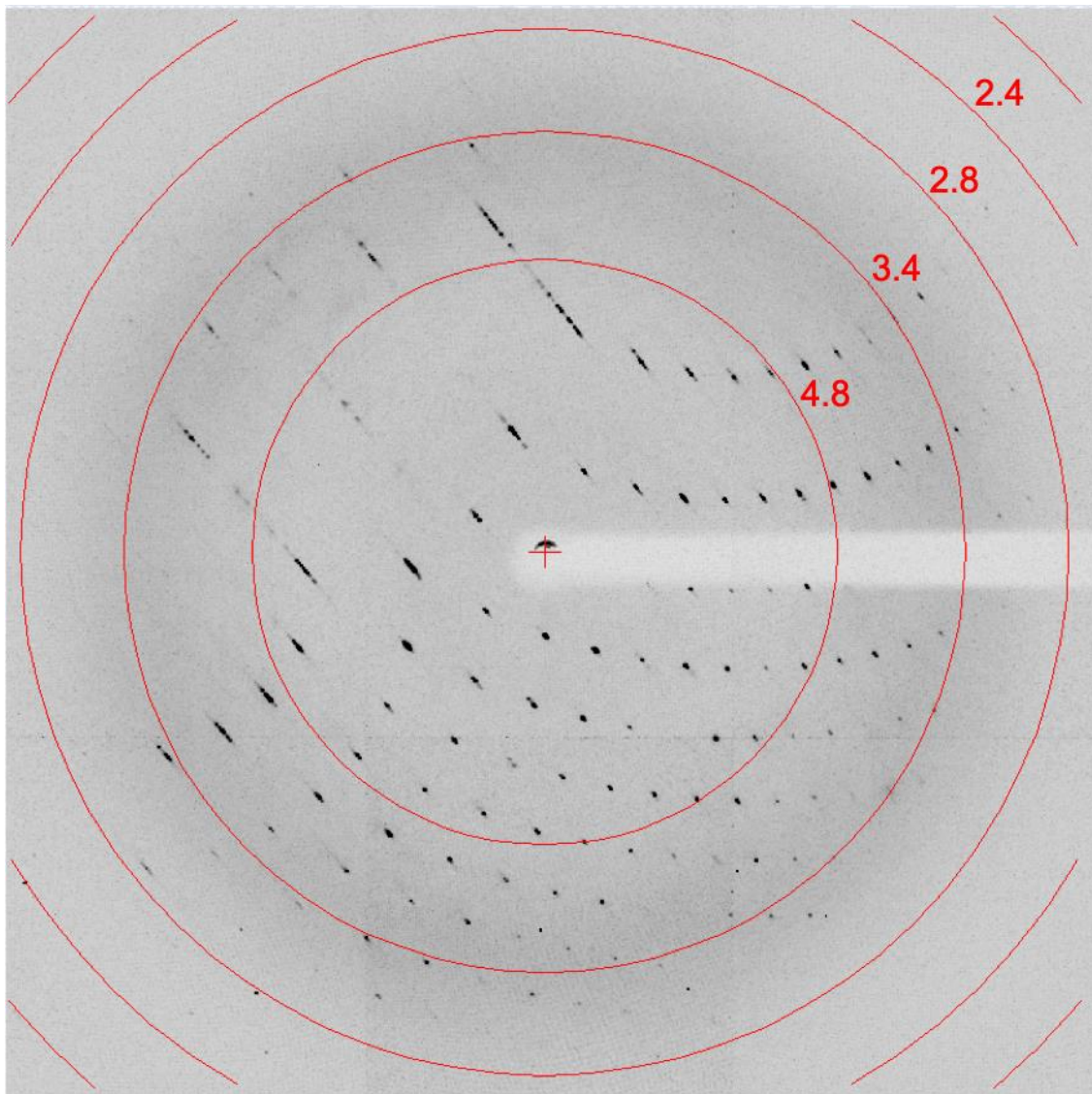
Supplementary Fig. S4: X-ray diffraction image of recombinant SpaB protein.
Diffraction pattern collected from a single crystal of Lys-methylated SpaB at the
synchrotron source. Resolution arcs (red circles) in Å are shown.

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              1        10        20            30        40        50
              .        .         .             .         .         .
SpaB   ...TVPTTVDVVLHKLLFKDTLPTQ.......QANNGTTKPDFSQADVPLNGVTFTVYDV
5FIE   ..TNDTTTQNVVLTKYGFDKDVT.........AIDRATDQIWTGDGAKPLQGVDFTIYNV
5Z24   ...AATTTVDFTLHKIEQTSDEQ...........IQNTGHDLGLTGRKPVQGAQFKIFNV
4HSS   ...DFERKGSLTLHKKKGAESE...........KRATGKEMDDVAGEPLNGVTFKITKL
6JCH   DQTAEIVIHKRIYRDIRQPEDVWYENDGHRIDPNNPDKDGYKLLSKTSGLNGANFEVYDA

                           60        70        80        90
                           .         .         .         .
SpaB   T...............ADFWQLVSKNGGAIEVAQTTLSQDSYQPASSSLIAQVVTAGQG
5FIE   T...............ANYW......ASPKDYKGSFDSAPVAATGTTNDKGQLTQALPI
5Z24   T...............DAFYQLLENHDKTTAASMISQNLGQYVNLQDPNAATVTTDADG
4HSS   N..............FDLQ.....NGDWAKFPKTAADAKGHETSTT...KEVETSGNG
6JCH   SSLLKPNMTPEAIRALVDRYQNMTRKQALKFARANLKLAGQGNKGLGLMNTKTDPTLGED

          100       110       120            130       140
          .         .         .              .         .
SpaB   EAYFGDLPLRQGQHAAVYLFKETAAP.......KNIEASQNLVVVMSSN..LQHGNQSRI
5FIE   QSKDASGKTR....AAVYLFHETNPR.......AGYNTSADFWLTLPAK....AADGNV
5Z24   LAAFKGLAAKTNGRHSVYAFHEAVTP.......QPYQKAADMIVSLPVR.QDDGSDLTNI
4HSS   TAVFDNLDLG......IYLVEETKAP.......DGIVTGAPFIVSIPMVNEASDAWNYNV
6JCH   GISRITVSVDQQAPTKAYLMIEVAPDPSTELNVDLERKSSPMLVVFPVTDPISGNPLQTI

          150
          .
SpaB   DLFPKN
5FIE   YVYPKN
5Z24   HLYPKD
4HSS   VAYPKN
6JCH   HLYPKN
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Supplementary Fig. S5: Multiple sequence alignment of *L. rhamnosus* GG basal SpaB pilin with other structurally known and closely related Gram-positive pilin subunits. SpaB amino acid sequence (see Table 1) served as the query input for a search of closest templates using the SWISS-MODEL server (https://swissmodel.expasy.org/). A ClustalW (https://embnet.vital-it.ch/software/ClustalW.html) multiple sequence alignment of the top five best hits includes the following pilin structures: *L. rhamnosus* GG SpaA N-domain (28% sequence identity, PDB ID: 5FIE); *L. rhamnosus* GG SpaD (26% sequence identity, PDB ID: 5Z24); *Corynebacterium diphtheriae* SpaD (25% sequence identity, PDB ID: 4HSS); and *L. rhamnosus* GG SpaE (21% sequence identity, PDB ID: 6JCH).