

Fig. S1

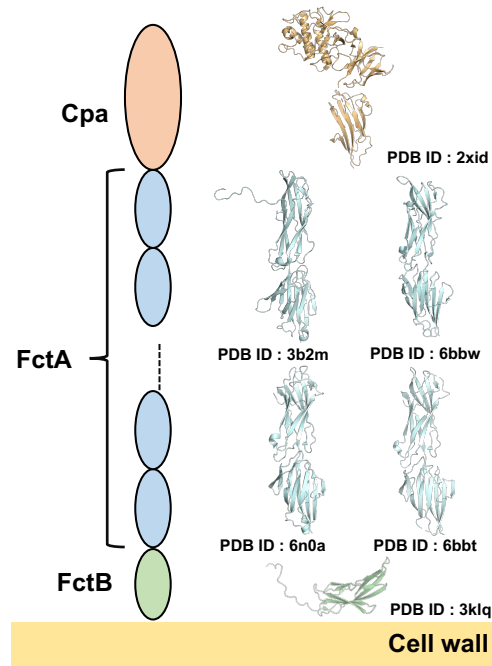


Fig. S1 Schema of pilin produced by *Streptococcus pyogenes* possessing FCT type 2-4 regions. The pilus shaft is composed of the major subunit FctA, while the minor subunit Cpa and FctB are pilus chip and base proteins, respectively. Shown followings are the PDB IDs (T serotypes) of previously reported crystal structures of pilins; FctA, 3B2M (T1), 6BBW (T3), 6N0A (T18), and 6BBT (T13); Cpa, 2XID (T1); FctB, PDB ID 3KLQ (T9).

Fig. S2

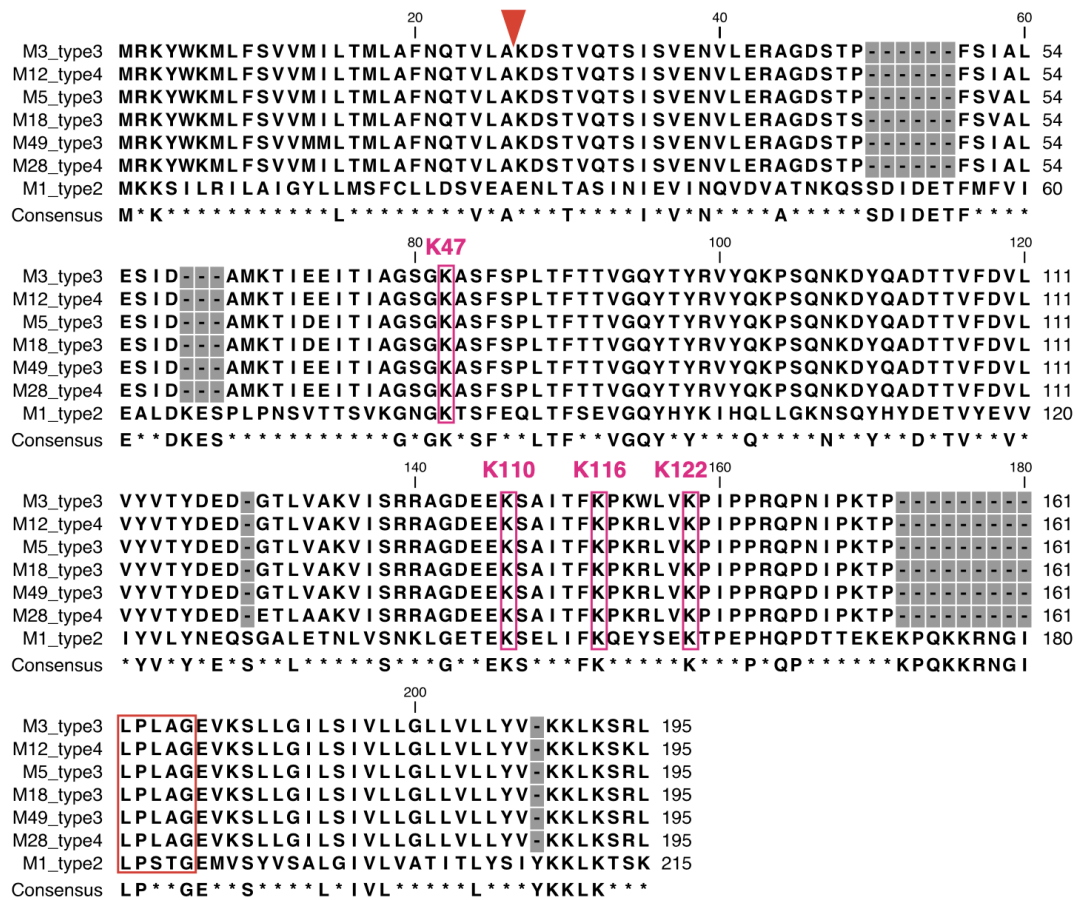


Fig. S2 Multiple sequence alignment of FctB from FCT region types 2/3/4. Alignment of FctB was performed using ClustalW. Shown following are the GenBank accession numbers of the utilized *S. pyogenes* DNA sequence and its M serotypes: NC002737, SF370, M1; NC004606, SSI-1, M3; NC009332, Manfredo, M5; AF447492, A735, M12; NC003485, MGAS8232, M18; CP000056, MGAS6180, M28; NZ_CP077685.1, 591, M49. Consensus residues conserved in all of the FctB sequence are also shown. LPLAG and LPSTG motifs are designated by orange outlining. The putative cleavage site by signal peptidase is indicated by the pink arrowhead. The positions of mutated lysine residues are shown by pink outlining.

Table S1 Plasmids and bacterial strains used in this study

Strain or plasmid	Property	Reference or source
Plasmid		
pQE30-FctB3	Expression vector for FctB3	This study
pSET4s	Temperature-sensitive shuttle vector	Takamatsu et al. 2001
pSET4s- <i>cpa</i> KO	Derivative of pSET4s for <i>cpa</i> deletion	This study
pSET4s- <i>sipA</i> KO	Derivative of pSET4s for <i>sipA</i> deletion	This study
pSET4s- <i>fctA</i> KO	Derivative of pSET4s for <i>fctA</i> deletion	This study
pSET4s- <i>srtC2</i> KO	Derivative of pSET4s for <i>srtC2</i> deletion	This study
pSET4s- <i>fctB</i> KO	Derivative of pSET4s for <i>fctB</i> deletion	This study
pSET4s- <i>fctB</i> KOinΔ <i>fctA</i>	Derivative of pSET4s for <i>fctB</i> deletion in Δ <i>fctA</i>	This study
pSET4s- <i>cpa</i> KOinΔ <i>fctA</i>	Derivative of pSET4s for <i>cpa</i> deletion in Δ <i>fctA</i>	This study
pSET4s- <i>sipA</i> KOinΔ <i>fctA</i> / <i>fctB</i>	Derivative of pSET4s for <i>sipA</i> deletion in Δ <i>fctA</i> / <i>fctB</i>	This study
pSET4s- <i>sipA</i> KOinΔ <i>cpa</i> / <i>fctB</i>	Derivative of pSET4s for <i>sipA</i> deletion in Δ <i>cpa</i> / <i>fctB</i>	This study
pAT18-PgyrA	Shuttle vector harboring <i>gyrA</i> promoter upstream of MCS	Nakata et al. 2009
pAT18-PgyrA- <i>fctB</i>	pAT18-PgyrA harboring wild-type <i>fctB</i>	Nakata et al. 2009
pAT18-PgyrA- <i>fctB</i> _K47A	pAT18-PgyrA harboring <i>fctB</i> K47A	This study
pAT18-PgyrA- <i>fctB</i> _K110A	pAT18-PgyrA harboring <i>fctB</i> K110A	This study
pAT18-PgyrA- <i>fctB</i> _K116A	pAT18-PgyrA harboring <i>fctB</i> K116A	This study
pAT18-PgyrA- <i>fctB</i> _K118A	pAT18-PgyrA harboring <i>fctB</i> K118A	This study
pAT18-PgyrA- <i>fctB</i> _K122A	pAT18-PgyrA harboring <i>fctB</i> K122A	This study
<i>Escherichia coli</i>		
XL10-Gold	Host for derivatives of pSET4s and pAT18	Stratagene
<i>Streptococcus pyogenes</i>		
591	M49 strain isolated from patient with pyoderma	Nakata et al. 2009
Δ <i>cpa</i>	In-frame <i>cpa</i> deletion mutant of 591	This study
Wr <i>cpa</i>	Revertant recovered during construction of Δ <i>cpa</i>	This study
Δ <i>sipA</i>	In-frame <i>sipA</i> deletion mutant of 591	This study
Wr <i>sipA</i>	Revertant recovered during construction of Δ <i>sipA</i>	This study
Δ <i>fctA</i>	In-frame <i>fctA</i> deletion mutant of 591	This study
Wr <i>fctA</i>	Revertant recovered during construction of Δ <i>fctA</i>	This study
Δ <i>srtC2</i>	In-frame <i>srtC2</i> deletion mutant of 591	This study
Wr <i>srtC2</i>	Revertant recovered during construction of Δ <i>srtC2</i>	This study
Δ <i>fctB</i>	In-frame <i>fctB</i> deletion mutant of 591	This study
Wr <i>fctB</i>	Revertant recovered during construction of Δ <i>fctB</i>	This study
Δ <i>srtA</i>	<i>srtA</i> deletion mutant of 591	Nakata et al. 2009
Δ <i>srtA</i> : <i>srtA</i>	Δ <i>srtA</i> complemented with pAT18-PgyrA- <i>srtA</i>	Nakata et al. 2009
Δ <i>fctB</i> Mock	Δ <i>fctB</i> transformed with pAT18-PgyrA	This study
Δ <i>fctB</i> :: <i>fctB</i>	Δ <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i>	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K47A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K47A	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K110A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K110A	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K116A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K116A	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K118A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K118A	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K122A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K122A	This study
Δ <i>cpa</i> / <i>fctB</i>	<i>cpa</i> / <i>fctB</i> deletion mutant of 591	This study
Δ <i>cpa</i> / <i>fctB</i> Mock	Δ <i>cpa</i> / <i>fctB</i> transformed with pAT18-PgyrA	This study
Δ <i>cpa</i> / <i>fctB</i> :: <i>fctB</i>	Δ <i>cpa</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i>	This study
Δ <i>cpa</i> / <i>fctB</i> :: <i>fctB</i> _K73A	Δ <i>cpa</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K73A	This study
Δ <i>cpa</i> / <i>fctB</i> :: <i>fctB</i> _K136A	Δ <i>cpa</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K136A	This study
Δ <i>fctA</i> / <i>fctB</i>	<i>fctA</i> / <i>fctB</i> deletion mutant of 591	This study
Δ <i>fctA</i> / <i>fctB</i> Mock	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i>	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i>	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K73A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K73A	This study
Δ <i>fctA</i> / <i>fctB</i> :: <i>fctB</i> _K136A	Δ <i>fctA</i> / <i>fctB</i> transformed with pAT18-PgyrA- <i>fctB</i> _K136A	This study

Table S1 continued

Strain or plasmid	Property	Reference or source
<i>Streptococcus pyogenes</i>		
$\Delta cpa/sipA/fctB$	<i>cpa/sipA/fctB</i> deletion mutant of 591	This study
$\Delta cpa/fctB$ WrsipA	Revertant recovered during <i>sipA</i> deletion	This study
$\Delta cpa/sipA/fctB$ Mock	$\Delta cpa/sipA/fctB$ transformed with pAT18-PgyrA	This study
$\Delta cpa/sipA/fctB::fctB$	$\Delta cpa/sipA/fctB$ transformed with pAT18-PgyrA- <i>fctB</i>	This study
$\Delta cpa/fctB$ WrsipA Mock	$\Delta cpa/fctB$ WrsipA transformed with pAT18-PgyrA	This study
$\Delta cpa/fctB$ WrsipA:: <i>fctB</i>	$\Delta cpa/fctB$ WrsipA transformed with pAT18-PgyrA- <i>fctB</i>	This study
$\Delta sipA/fctA/fctB$	<i>cpa/sipA/fctB</i> deletion mutant of 591	This study
$\Delta fctA/fctB$ WrsipA	Revertant recovered during <i>sipA</i> deletion	This study
$\Delta cpa/sipA/fctB$ Mock	$\Delta cpa/sipA/fctB$ transformed with pAT18-PgyrA	This study
$\Delta cpa/sipA/fctB::fctB$	$\Delta cpa/sipA/fctB$ transformed with pAT18-PgyrA- <i>fctB</i>	This study
$\Delta cpa/fctB$ WrsipA Mock	$\Delta cpa/fctB$ WrsipA transformed with pAT18-PgyrA	This study
$\Delta cpa/fctB$ WrsipA:: <i>fctB</i>	$\Delta cpa/fctB$ WrsipA transformed with pAT18-PgyrA- <i>fctB</i>	This study

Table S2 Primers used in this study

Primer	Sequence (5'-3')
cpaKO F1	GCGGATCCGACTCTCAAGCCAAATTCCCGTA
cpaKO R1	ATAACCCAAGTGGAAACAAGCAATATATCCTCTCCTTCC
cpaKO F2	GGAAGGAGAGGATATATTGCTTGTTCACCTGGGGTAT
cpaKO R2	GCCTGCAGCCATCAATCACTCCTGGCTTAAT
sipAKO F1	GCGGATCCGCGCTAAGACCGAGAGATACAAA
sipAKO R1 ^{1,2}	TCCTCTCACTCTTAATAGAGTACGATTTAGGTAATTAGTCAT
sipAKO F2 ^{1,2}	ATGACTAATTACCTAAATCGTACTCTATTAAGAGTGAGAGGA
sipAKO R2 ^{1,2}	GCCTGCAGTCAGTTTTATGGTCTTTACCTAAT
fctAKOF1	GCGGATCCGGACAGCCTATTGTTAACACTCT
fctAKOR1	TAATATAGATAACTCCACCCATTTTTCTCTCTCCAAA
fctAKOF2	TTTGGAGAGAGAAAAAATGGGTGGAGTTATCTATATTA
fctAKOR2	GCCTGCAGCAGTCTGATTAAAGGCCAGCATG
srtC2KOF1	GCGGATCCGGATGATAACGTTTTAATGCCAA
srtC2KOR1	TCGTTATTCTTGAATAGTACCCATCATCATTCTTTGACTAAT
srtC2KOF2	ATTAGTCAAAGAATGATGATGGGTACTATTCAAGAATAACGA
srtC2KOR2	GCCTGCAGGGAATTCCTGTTGGAGAAGTGGCT
fctBKOF1	GCGGATCCGTTGTAGGCACCCTTGCTCCA
fctBKOR1 ³	CTCTTAAAAATGGGTTTTACAAAAGTCTCCTCCTTTTCG
fctBKOF2 ³	CGAAAGGAGGAGACTTTTTGTAAACCCATTTTTAAGAG
fctBKOR2 ³	GCCTGCAGCCTGATATCATTATCGCCTT
cpaKOR2forFctAKO	GCCTGCAGGAGTCCCTGGGATATTTAACCAA
sipKOF1forCpa/FctBKO ¹	GCGGATCCGATTCCCGTAGTCGATAAGCTGA
sipKOR2forFctA/FctBKO ²	GCCTGCAGCGTTATTCTTGAATAGTACCGA
fctBKOF1forFctAKO ³	GCGGATCCGTGATTAGTCAAAGAATGATGAT
pSET4scheckF ⁴	GCAAGGCGATTAAAGTTGGGTAA
pSET4scheckR ⁴	GGAATTGTGAGCGGATAACAAT
fctBcompF ⁵	GGCGGATCCTTGAGAAAATATTGGAAAATGT
fctBcompR ⁵	GGCCTGCAGTTATAGCCTACTCTTCAGTTTTTTTA
fctBK73A ⁵	GGAAAAGCTTGCTGCTCCAGAACC
fctBK136A ⁵	AGTAATCGCTGATGCTTCTTCGTC
fctBK142A ⁵	CCGTTTGGGTGCAAAAGTAATCGC
fctBK144A ⁵	TACTAACCGTGCGGGCTTAAAAGT
fctBK148A ⁵	CGGTATTGGTGCTACTAACCCTTT
mutt1 ⁴	CATGATTACGAGTTCTAGCT
M13_M4 ⁴	GTTTTCCAGTCACGAC
M13_RV ⁴	CAGGAAACAGCTATGAC
rFctB3F ⁶	GCCGGATCCAAAGACAGCACTGTTCAAACCT
rFctB3R ⁶	GCCCTGCAGTTAAGCTAATGGTAATGGGGTT
pQEForward ⁴	CGGATAACAATTTACACAG
pQEREverse ⁴	GTTCTGAGGTCATTACTGG

¹; Used for the construction of Δ cpa/sipA/fctB²; Used for the construction of Δ sipA/fctA/fctB³; Used for the construction of Δ fctA/fctB⁴; Used for sequencing and colony direct PCR⁵; Used for the construction of expression vectors of wild-type and mutant *fctB*⁶; Used for the construction of expression vectors of recombinant FctB3

Table S3 Crystallization conditions

Method	Vapor diffusion, sitting drop
Plate type	Sitting-drop vapor-diffusion plate
Temperature (K)	293
Protein concentration (mg ml ⁻¹)	45.13
Buffer composition of protein solution	50 mM Tris-HCl, pH 8.0, 150 mM NaCl
Composition of reservoir solution	1.8 M ammonium sulfate, 0.1 M sodium acetate trihydrate, pH 4.2
Volume (μl) and ratio of drop	2 (1:1)
Volume of reservoir (μl)	500