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Supplementary Materials for

Structure and mechanism for streptococcal fatty acid kinase (Fak) system dedicated to host fatty acid scavenging

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Table S1 Data collection and refinement statistics of *S. suis* FakA complexed with its partner FakB2

Data Set	SsFakA/FakB2
Data collection	
Wavelength (Å)	0.9792
Space group	*P 43 21 2*
a, *b*, *c* (Å)	107.75, 107.75, 399.0
a, *b*, *g* (°)	90, 90, 90
Resolution (Å)	45 -- 2.6 (2.693 -- 2.6)
Observed reflections	634,580 (62,095)
Unique reflections	72,275 (7232)
R_{merge} (%)	7.868 (119.2)
R_{pim} (%)	2.851 (42.92)
$I/\sigma(I)$	12.13 (1.52)
$CC_{1/2}$	0.999 (0.84)
Completeness (%)	99.49 (99.92)
Wilson B-factor (Å^2)	77.47
Multiplicity	8.7 (8.6)
Refinement	
R_{work}/R_{free} (%)	18.24/22.06
No. protein atoms	10,285
No. ligand atoms	47
Average B-factor (Å^2)	90.76
Protein B-factor (Å^2)	90.73
Ligand B-factor (Å^2)	98.99
Rmsd bond lengths (Å)	0.009
Rmsd bond angles (°)	1.17
Ramachandran outliers (%)	0
Ramachandran favored (%)	96.88

Values in parentheses are for the highest-resolution shell.

Table S2 Bacterial strains and plasmids used in this study

Strains/plasmids	Description	Origins
Strains		
05ZYH33	An epidemic strain of Chinese virulent *S. suis* serotype 2	Lab stock
FYJ902	Δ*fakB*1 mutant of 05ZYH33	This work
FYJ903	Δ*fakB*2 mutant of 05ZYH33	This work
DH5α	A cloning host of *E. coli*	Lab stock
BL21	An expression host of *E. coli*	Lab stock
FYJ582	BL21 carrying pET28a*::aasS*	Lab stock
FYJ571	BL21 carrying pET28a*::fakA*	This work
FYJ572	BL21 carrying pET28a*::fakB1*	This work
FYJ573	BL21 carrying pET28a*::fakB2*	This work
FYJ5100	BL21 carrying pET21a*::fakB3*	This work
FYJ5101	BL21 carrying pET28a*::fakA*(E538A)	This work
FYJ5102	BL21 carrying pET28a*::fakA*(E554K)	This work
FYJ5103	BL21 carrying pET28a*::fakB2*(R170A)	This work
FYJ5104	BL21 carrying pET28a*::fakB2*(L181D)	This work
FYJ5105	BL21 carrying pET28a*::fakB2*(R202A)	This work
FYJ5106	BL21 carrying pET28a*::fakA*(D37A)	This work
FYJ5107	BL21 carrying pET28a*::fakA*(D39A)	This work
FYJ5108	BL21 carrying pET28a*::fakA*(D39G)	This work
FYJ5109	BL21 carrying pET28a*::fakA*(D39R)	This work
FYJ5110	BL21 carrying pET28a*::fakA*(K125A)	This work
FYJ5111	BL21 carrying pET28a*::fakA*(K395A)	This work
FYJ5112	BL21 carrying pET28a*::fakA*(K418A)	This work
FYJ5113	BL21 carrying pET28a*::fakA*(K481A)	This work
FYJ5114	BL21 carrying pET28a*::fakA*(K125A/K395A)	This work
FYJ5115	BL21 carrying pET28a*::fakA*(K125A/K418A)	This work
FYJ5116	BL21 carrying pET28a*::fakA*(K125A/K481A)	This work
FYJ5117	BL21 carrying pET28a*::fakA*(K395A/K418A)	This work
FYJ5118	BL21 carrying pET28a*::fakA*(K395A/K481A)	This work
FYJ5119	BL21 carrying pET28a*::fakA*(K418A/K481A)	This work
FYJ5120	BL21 carrying pET28a*::fakA* (K125A/K395A/K418A)	This work
FYJ5121	BL21 carrying pET28a*::fakA* (K125A/K418A/K481A)	This work
FYJ5122	BL21 carrying pET28a*::fakA* (K395A/K418A/K481A)	This work
FYJ5123	BL21 carrying pET28a*::fakA* (K125A/K395A/K481A)	This work
FYJ5124	BL21 carrying pET28a*::fakA* (K125A/K395A/K418A/K481A)	This work

FYJ5125	BL21 carrying pET28a::*fakB1*(R172A)	This work
FYJ5126	BL21 carrying pET28a::*fakB1*(L183D)	This work
FYJ5127	BL21 carrying pET28a::*fakB1*(R205A)	This work
FYJ5128	BL21 carrying pET21a::*fakB3*(R174A)	This work
FYJ5129	BL21 carrying pET21a::*fakB3*(L185D)	This work
FYJ5130	BL21 carrying pET21a::*fakB3*(R207A)	This work
FYJ5131	BL21 carrying pET21a::*fakA*_N	This work
FYJ5132	BL21 carrying pET21a::*fakA*_M	This work
FYJ5133	BL21 carrying pET28a::*fakA*_C	This work
FYJ5134	BL21 carrying pET28a::*plsX*	This work
FYJ5135	BL21 carrying pET28a::*fakA*(D39E)	This work
FYJ5136	BL21 carrying pET28a::*fakA*(D39V)	This work
FYJ5137	BL21 carrying pET28a::*fakA*(D39S)	This work
FYJ5138	BL21 carrying pET28a::*fakA*(D39L)	This work
FYJ5139	BL21 carrying pET28a::*fakA*(H226A)	This work
FYJ5140	BL21 carrying pET28a::*fakA*(C244A)	This work
FYJ5141	BL21 carrying pET28a::*fakA*(H289A)	This work
FYJ5142	BL21 carrying pET28a::*fakA*(H291A)	This work
Plasmids		
pUC19-*Spc*	A derivative of pUC19 for in-frame gene deletion from *S. suis* genome, Spc^R, Amp^R	Lab stock
pUC19::*fakB*1-LSR	A knock-out plasmid for *S. suis fakB1* gene	This work
pUC19::*fakB*2-LSR	A knock-out plasmid for *S. suis fakB2* gene	This work
pET21a	A T7 promoter-driven expression vector, Amp^R	Lab stock
pET28a	A T7 promoter-driven expression vector, Km^R	Lab stock
pET28a::*fakA*	pET28a encoding *fakA*, Km^R	This work
pET28a::*fakB1*	pET28a encoding *fakB1*, Km^R	This work
pET28a::*fakB2*	pET28a encoding *fakB2*, Km^R	This work
pET21a::*fakB3*	pET21a encoding *fakB3*, Amp^R	This work
pET28a::*fakA*(E538A)	pET28a encoding the mutant version of *fakA*(E538A), Km^R	This work
pET28a::*fakA*(E554K)	pET28a encoding the mutant version of *fakA*(E554K), Km^R	This work
pET28a::*fakB2*(R170A)	pET28a encoding the mutant version of *fakB2*(R170A), Km^R	This work
pET28a::*fakB2*(L181D)	pET28a encoding the mutant version of *fakB2*(L181D), Km^R	This work
pET28a::*fakB2*(R202A)	pET28a encoding the mutant version of *fakB2*(R202A), Km^R	This work
pET28a::*fakA*(D37A)	pET28a encoding the *fakA*(D37A) mutant, Km^R	This work
pET28a::*fakA*(D39A)	pET28a encoding the mutant version of *fakA*(D39A), Km^R	This work

pET28a::*fakA*(D39G)	pET28a encoding the mutant version of *fakA*(D39G), Km^R	This work
pET28a::*fakA*(D39R)	pET28a encoding the mutant version of *fakA*(D39R), Km^R	This work
pET28a::*fakA*(D39E)	pET28a carrying the *fakA*(D39E) mutant, Km^R	This work
pET28a::*fakA*(D39V)	pET28a carrying the *fakA*(D39V) mutant, Km^R	This work
pET28a::*fakA*(D39S)	pET28a carrying the *fakA*(D39S) mutant, Km^R	This work
pET28a::*fakA*(D39L)	pET28a carrying the *fakA*(D39L) mutant, Km^R	This work
pET28a::*fakA*(H226A)	pET28a carrying the *fakA*(H226A) mutant, Km^R	This work
pET28a::*fakA*(C244A)	pET28a carrying the *fakA*(C244A) mutant, Km^R	This work
pET28a::*fakA*(H289A)	pET28a carrying the *fakA*(H289A) mutant, Km^R	This work
pET28a::*fakA*(H291A)	pET28a carrying the *fakA*(H291A) mutant, Km^R	This work
pET28a::*fakA*(K125A)	pET28a encoding the mutant version of *fakA*(K125A), Km^R	This work
pET28a::*fakA*(K395A)	pET28a encoding the mutant version of *fakA*(K395A), Km^R	This work
pET28a::*fakA*(K418A)	pET28a encoding the mutant version of *fakA*(K418A), Km^R	This work
pET28a::*fakA*(K481A)	pET28a encoding the mutant version of *fakA*(K481A), Km^R	This work
pET28a::*fakA*(K125A/K395A)	pET28a encoding the mutant version of *fakA*(K125A/K395A), Km^R	This work
pET28a::*fakA*(K125A/K418A)	pET28a encoding the mutant version of *fakA*(K125A/K418A), Km^R	This work
pET28a::*fakA*(K125A/K481A)	pET28a encoding the mutant version of *fakA*(K125A/K481A), Km^R	This work
pET28a::*fakA*(K395A/K418A)	pET28a encoding the mutant version of *fakA*(K395A/K418A), Km^R	This work
pET28a::*fakA*(K395A/K481A)	pET28a encoding the mutant version of *fakA*(K395A/K481A), Km^R	This work
pET28a::*fakA*(K418A/K481A)	pET28a encoding the mutant version of *fakA*(K418A/K481A), Km^R	This work
pET28a::*fakA*(K125A/K395A/K418A)	pET28a encoding the mutant version of *fakA*(K125A/K395A/K418A), Km^R	This work
pET28a::*fakA*(K125A/K418A/K481A)	pET28a encoding the mutant version of *fakA*(K125A/K418A/K481A), Km^R	This work
pET28a::*fakA*(K395A/K418A/K481A)	pET28a encoding the mutant version of *fakA*(K395A/K418A/K481A), Km^R	This work
pET28a::*fakA*(K125A/K395A/K481A)	pET28a encoding the mutant version of *fakA*(K125A/K395A/K481A), Km^R	This work
pET28a::*fakA*(K125A/K395A/K418A/K481A)	pET28a encoding the mutant version of *fakA*(K125A/K395A/K418A/K481A), Km^R	This work
pET28a::*fakB1*(R172A)	pET28a encoding the mutant version of	This work

	fakB1(R172A), Km^R	
pET28a::*fakB1*(L183D)	pET28a encoding the mutant version of *fakB1*(L183D), Km^R	This work
pET28a::*fakB1*(R205A)	pET28a encoding the mutant version of *fakB1*(R205A), Km^R	This work
pET21a::*fakB3*(R174A)	pET21a encoding the mutant version of *fakB3*(R174A), Amp^R	This work
pET21a::*fakB3*(L185D)	pET21a encoding the mutant version of *fakB3*(L185D), Amp^R	This work
pET21a::*fakB3*(R207A)	pET21a encoding the mutant version of *fakB3*(R207A), Amp^R	This work
pET21a::*fakA*_N	pET21a encoding *fakA*_N, Amp^R	This work
pET21a::*fakA*_M	pET21a encoding *fakA*_M, Amp^R	This work
pET28a::*fakA*_C	pET28a encoding *fakA*_C, Km^R	This work
pET28a::*plsX*	pET28a encoding *plsX*, Km^R	This work

Table S3 Primers used in this study

Primers	Sequences
fakB1-F	5’-ATG AAA TTA GCG GTT ATA ACT GAC T-3’
fakB1-R	5’-CTA TTC AAT AAT GGG AGA AAT ACC A-3’
fakB1-P-F	5’-CGT GTT TTG GTA GAG CCT CTT TT-3’
fakB1-P-R	5’-AGC CAG ACT AAG TGC TGC GTC-3’
fakB2-F	5’-ATG TCA AAG ATT AAG ATT GTT ACG G-3’
fakB2-R	5’-TTA TTC ATA ACG AAG CAA AAT AGC C-3’
fakB2-P-F	5’-TAG GAA GTT GAT TCA AGG CTT TGT-3’
fakB2-P-R	5’-ATT TCA TCC TAC AAT TCT CTT CAA TT-3’
Spc-F	5’-GTT CGT GAA TAC ATG TTA TAA TAA C-3’
Spc-R	5’-GTT TTC TAA AAT CTG ATT ACC AAT T-3’
fakA(E538A)-F	5'-TGA GGT C**GC A**AT CCA TCA AGG AAA TCA ACC AGT C-3'
fakA(E538A)-R	5'-GAT GGA T**TG C**GA CCT CAA CAT CTT CAA ATT GTT CTT-3'
fakA(E554K)-F	5'-CAG CGT A**AA G**TA ACT CGA GCA CCA CCA CCA CC-3'
fakA(E554K)-R	5'-CGA GTT A**CT T**TA CGC TGA AAA GAT ATG GAT AGA CTG G-3'
fakB2(R170A)-F	5'-AGG GTG GT**G CA**A TTG GAC GTG TTC AAG GGA TGC-3'
fakB2(R170A)-R	5'-TCC AAT **TGC** ACC ACC CTT GAC TAG ATT TTC TAG GG-3'
fakB2(L181D)-F	5'-GAG TAG C**GA C**CT CAA TAT CCG TGT CAT CAT GGA A-3'
fakB2(L181D)-R	5'-TAT TGA G**GT C**GC TAC TCA GCA TCC CTT GAA CAC-3'
fakB2(R202A)-F	5'-CAA GGG A**GC A**GG CAA TAA GAC CTT TAA AAA ATG GC-3'
fakB2(R202A)-R	5'-TAT TGC C**TG C**TC CCT TGA CAA TCG GAG TCA GC-3'
fakA(D37A)-F	5'-TTT TCC CAG TTC CG**G CA**G GGG ATA CTG GTA CCA ACA TGG-3'
fakA(D37A)-R	5'-**TGC** CGG AAC TGG GAA AAC ATT GAG TGA GTT CA-3'
fakA(D39A)-F	5'-G**GC A**AC TGG TAC CAA CAT GGG GAT GAC TAT TA-3'
fakA(D39A)-R	5'-TGT TGG TAC CAG T**TG C**CC CAT CCG GAA CTG GGA A-3'
fakA(D39G)-F	5'-G**GG A**AC TGG TAC CAA CAT GGG GAT GAC TAT TA-3'
fakA(D39G)-R	5'-TGT TGG TAC CAG T**TC C**CC CAT CCG GAA CTG GGA A-3'
fakA(D39R)-F	5'-GGA TGG G**AG A**AC TGG TAC CAA CAT GGG GAT GA-3'
fakA(D39R)-R	5'-TAC CAG T**TC T**CC CAT CCG GAA CTG GGA AAA CA-3'
fakA(D39E)-F	5'-GGA TGG G**GA A**AC TGG TAC CAA CAT GGG GAT GA-3’
fakA(D39E)-R	5'-TAC CAG T**TT C**CC CAT CCG GAA CTG GGA AAA CA-3’
fakA(D39V)-F	5'-GGA TGG G**GT A**AC TGG TAC CAA CAT GGG GAT GA-3’
fakA(D39V)-R	5'-TAC CAG T**TA C**CC CAT CCG GAA CTG GGA AAA CA-3’
fakA(D39S)-F	5'-G**AG C**AC TGG TAC CAA CAT GGG GAT GAC TAT TA-3’
fakA(D39S)-R	5'-TGT TGG TAC CAG T**GC T**CC CAT CCG GAA CTG GGA A-3’
fakA(D39L)-F	5'-GGA TGG G**CT A**AC TGG TAC CAA CAT GGG GAT GA-3’
fakA(D39L)-R	5'-TAC CAG T**TA G**CC CAT CCG GAA CTG GGA AAA CA-3’
fakA(H226A)-F	5'-TGC TGA G**GC A**CA CAA GTC AGT TGC AGG TCA TGT-3’
fakA(H226A)-R	5'-ACT TGT G**TG C**CT CAG CAT TGA TCA TTT CAG ACA TAA-3’
fakA(C244A)-F	5'-CTT TGG TTA C**GC A**AC AGA AAT CAT GGT AGC TCT TCG C-3’
fakA(C244A)-R	5'-CTG T**TG C**GT AAC CAA AGG TAA TAT CTT CAG TCG C-3’
fakA(H289A)-F	5'-AAA GTC **GCA** GTC CAT ACA GAA GAT CCA GGT TTG G-3’
fakA(H289A)-R	5'-GTA TGG AC**T GC**G ACT TTA ACG ATT TCA TCA TCG TTG-3’

fakA(H291A)-F	5'-CCA TGT C**GC A**AC AGA AGA TCC AGG TTT GGT CAT G-3’
fakA(H291A)-R	5'-CTT CTG T**TG C**GA CAT GGA CTT TAA CGA TTT CAT CA-3’
fakA(K125A)-F	5'-CGT TAT G**GC A**CC AGT TGA AGG AAC AAT TCT TAC TGT-3'
fakA(K125A)-R	5'-CAA CTG G**TG C**CA TAA CGG CTT TAT AAG CCA CTT C-3'
fakA(K395A)-F	5'-GTT GCC AAA CAA C**GC A**AA TAT CTT GAT GGC TGC TCA GAC G-3'
fakA(K395A)-R	5'-T**TG C**GT TGT TTG GCA ACA AGA TGA CAT TGC GG-3'
fakA(K418A)-F	5'-GGA AAC T**GC A**AC CCT ACC ACA AGG TTT TAC TAG CTT-3'
fakA(K418A)-R	5'-GTA GGG T**TG C**AG TTT CCA CAA CCT TAA CCG CA-3'
fakA(K481A)-F	5'-TGA TGG C**GC A**AT TGT GGT TTC TAA TCC AGA TAT GAT G-3'
fakA(K481A)-F	5'-CCA CAA T**TG C**GC CAT CAA CCA TAC CAA GGT TG-3'
fakB1(R172A)-F	5'-AGG TGG T**GC A**TT GTC CAA TGG TGC AGC CTT GC-3'
fakB1(R172A)-R	5'-TGG ACA A**TG C**AC CAC CTT TTA CCA AAT GAT TGA GG-3'
fakB1(L183D)-F	5'-TGG CAA T**GA C**TT GAG CAT CAA ACC GAT TCT GTA-3'
fakB1(L183D)-R	5'-TGC TCA A**GT C**AT TGC CAA GCA AGG CTG CAC CA-3'
fakB1(R205A)-F	5'-AAG TT**G CA**A CGG AGA AGA AGG CCA TTA AAC GC-3'
fakB1(R205A)-R	5'-CTT CTC CGT **TGC** AAC TTT TTC ATA CAC TTC TAT CTT ACC CTC-3'
fakB3(R174A)-F	5'-GAA TGG A**GC A**CT GAG CAA ATT GGT AGG CAC TG-3'
fakB3(R174A)-R	5'-TGC TCA G**TG C**TC CAT TCT TAA CAA GAT TAT CAA CTT TCG-3'
fakB3(L185D)-F	5'-CGT TGG T**GA C**CT CAA TAT CCG TAT GGT TGG TGA G-3'
fakB3(L185D)-R	5'-TAT TGA G**GT C**AC CAA CGA CAG TGC CTA CCA AT-3'
fakB3(R207A)-F	5'-AAA GGC G**GC A**GG TCA TAA GAA ATC TGT GAC AGC AG-3'
fakB3(R207A)-R	5'-TAT GAC C**TG C**CG CCT TTT GAA GCA ACT CTA ATT-3'
pET21a-*fakA*_N-F	5'-GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GTG TCT AAA ATT ACA ACT AGT TTA-3'
pET21a-*fakA*_N-R	5'-ATT TCA GTG GTG GTG GTG GTG GTG CTC GAG ACC CGT CAA AGC TGA CAA GA-3'
pET21a-*fakA*_M-F	5'-GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT GTT ATG TCT GAA ATG ATC A-3'
pET21a-*fakA*_M-R	5'-ATT TCA GTG GTG GTG GTG GTG GTG CTC GAG CTG AGC TTC ATG TTG CTC AC-3'
pET28a-*fakA*_C-F	5'-CTG GTG CCG CGC GGC AGC CAT ATG GGA TCC AAA CCG GCT GAA CAA AAA GAG-3'
pET28a-*fakA*_C-R	5'-TCA GTG GTG GTG GTG GTG GTG CTC GAG TTA TTC TAC GCT GAA AAG ATA TGG A-3'
pET28a-*plsX*-F	5'-GGT GCC GCG CGG CAG CCA TAT GGG ATC CAT GAA ACG TAT TGC AGT AGA TG-3'
pET28a-*plsX*-R	5'-ATT TCA GTG GTG GTG GTG GTG GTG CTC GAG CTA GTC ATG ACT CTC CTC CA-3'

The codons with a point mutation are indicated with bold letters.

Supplementary figures

A

Species	FakB1	FakB2	FakB3	FakB4	FakB5
S. aureus Newman	100%	100%			
S. aureus MRSA252	100%	98.9%			
S. pneumoniae TIGR4	32.9%	27.8%	100%		
S. suis 05ZYH33	31.1%	29.0%	68.6%	100%	
S. suis P1/7	31.1%	29.0%	68.6%	100%	
S. pyogenes M1 GAS	33.9%	28.8%	63.3%	57.1%	
S. agalactiae 2603V/R	28.0%	30.2%		57.5%	
E. faecalis V583	37.7%	35.0%		39.0%	100%
E. faecium SRR24	36.0%	37.1%		38.6%	70.3%
L. lactis IL1403	31.1%	32.2%		47.8%	
L. fermentum IFO3956		31.4%	40.8%		

B

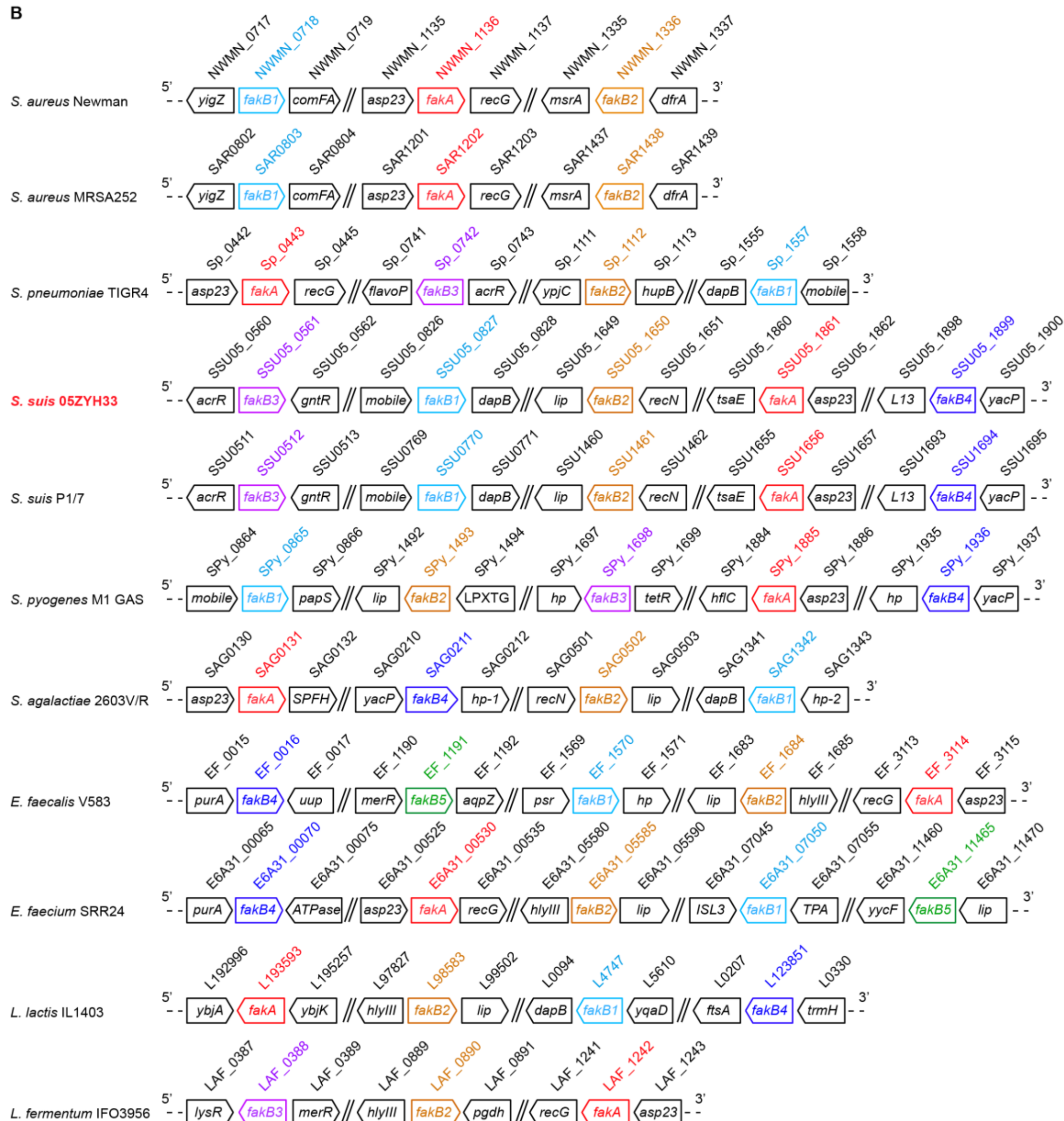


Fig. S1 Distribution and genetic organization of the FakA-FakB system

A. The collection of five FakB isoenzymes

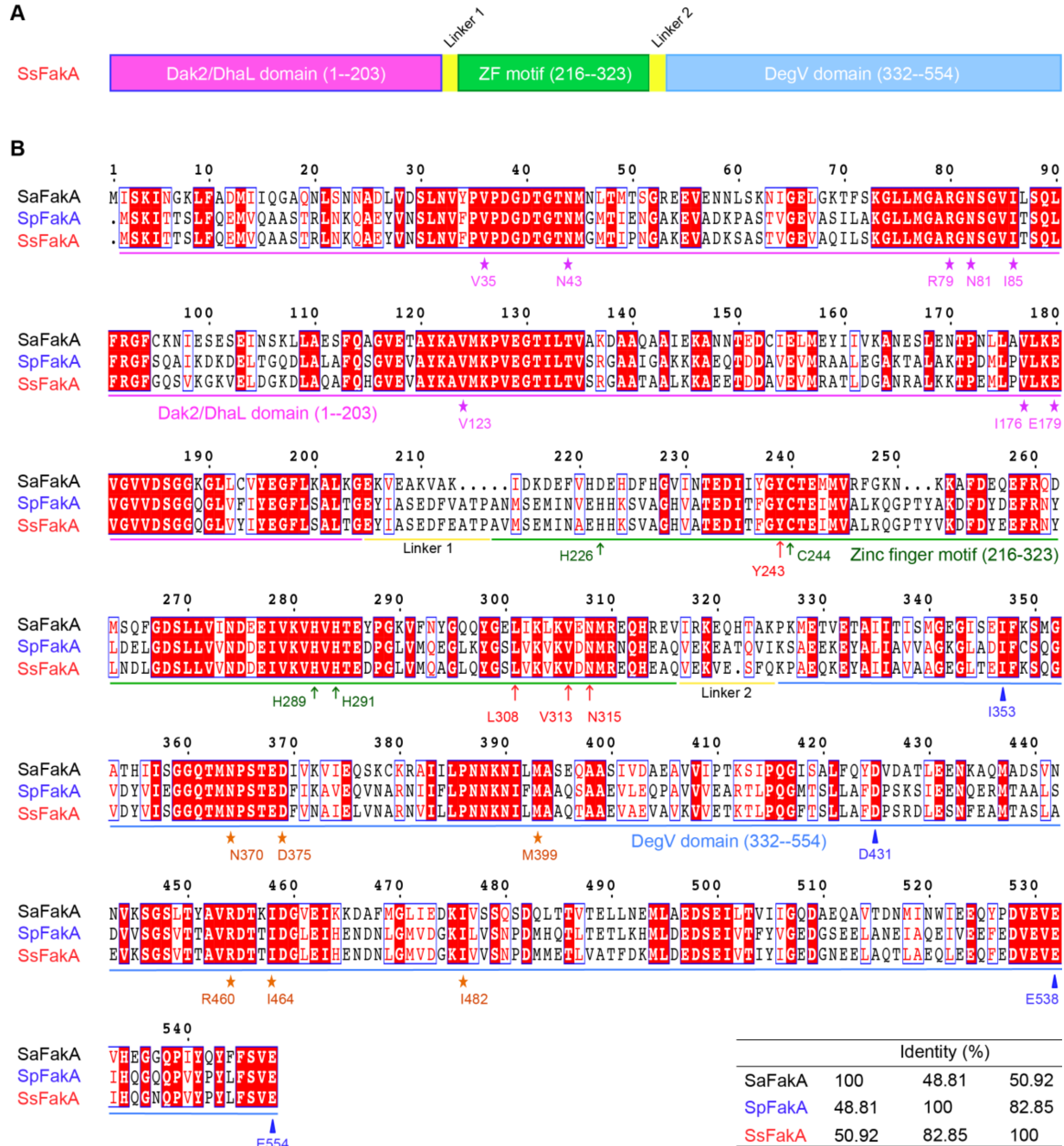
Five distinct isoforms of FakB are distributed in certain species of *Staphylococcus* and *Streptococcus*. The protein identities were calculated with the software Vector NTI, which might vary slightly when compared with the results of Clustal Omega-based alignment.

Designations: *S. aureus*, *Staphylococcus aureus*; *S. pneumoniae*, *Streptococcus pneumoniae*; *S. suis, Streptococcus suis*; *S. pyogenes*, *Streptococcus pyogenes*; *S. agalactiae*, *Streptococcus agalactiae*; *E. faecalis*, *Enterococcus faecalis*; *Enterococcus faecium*, *E. faecium*; *L. lactis*, *Lactococcus lactis*; *L. fermentum*, *Lactococcus fermentum*.

B. Genetic context of *fakA* and its related *fakB* isoforms

The double slash "//" denotes the discontinuity of two genes on a given chromosome.

The genome sequences of 11 bacterial species were sampled from Genebank database, namely i) AP009351 for *S. aureus* Newman; ii) BX571856 for *S. aureus* MRSA252; iii) NC_003028 for *S. pneumoniae* TIGR4; iv) CP000407 for *S. suis* 05ZYH33; v) AM946016 for *S. suis* P1/7; vi) AE004092 for *S. pyogenes* M1 GAS; vii) AE009948 for *S. agalactiae* 2603V/R; viii) AE016830 for *E. faecalis* V583; ix) CP038996 for *E. faecium* SRR24; x) AE005176 for *L. lactis* IL1403; and xi) AP008937 for *L. fermentum* IFO3956.



		Identity (%)	
SaFakA	100	48.81	50.92
SpFakA	48.81	100	82.85
SsFakA	50.92	82.85	100

Fig. S2 Bioinformatic analysis of FakA

A. Linear scheme for *Streptococcus suis* FakA

B. Sequence alignment of *S. suis* FakA with the two homologs from *Staphylococcus aureus* and *S. pneumoniae*

Multiple sequence alignment of FakA proteins was performed with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo), and the resultant output was used as input files for the program ESPript (https://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi).

Identical residues are white letters in red background; similar residues are red letters in white background; varied residues are dark letters. The protein identities are summarized in an inside table.

Designations: α, α-helices; β, β-sheets; T, turns; Sa, *Staphylococcus aureus*; Sp, *Streptococcus pneumoniae*; Ss, *Streptococcus suis* 2 (*S. suis* 2)

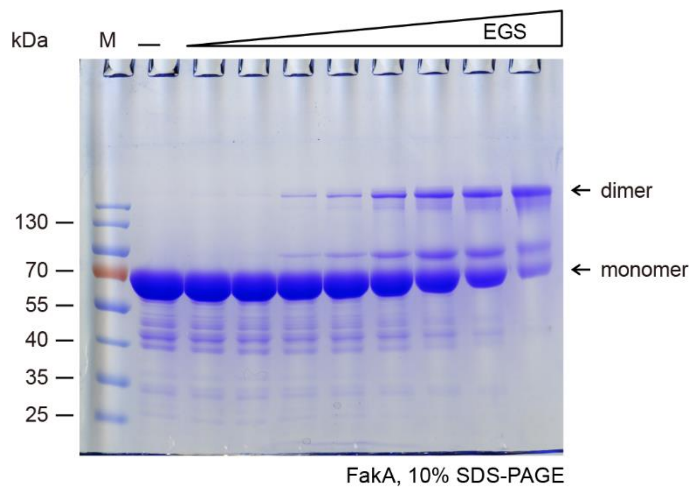


Fig. S3 Chemical cross-linking assay suggested that *Streptococcus suis* FakA is a dimer

The minus "--" denotes no addition of EGS cross-linker. The top triangle on the right hand (from left to right) refers to the addition of EGS at the following concentrations: 5, 10, 50, 100, 200, 500, 1000, 2000μM.

Abbreviations: EGS, Ethylene glycol bis (succinimidylsuccinate); M, protein marker; kDa, kilodalton.

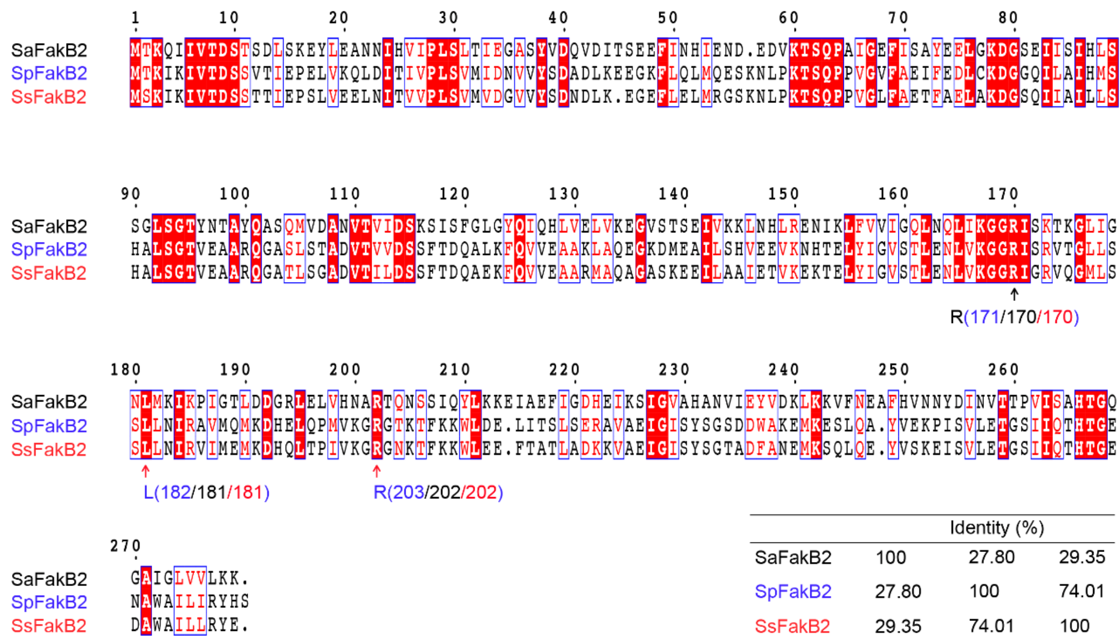
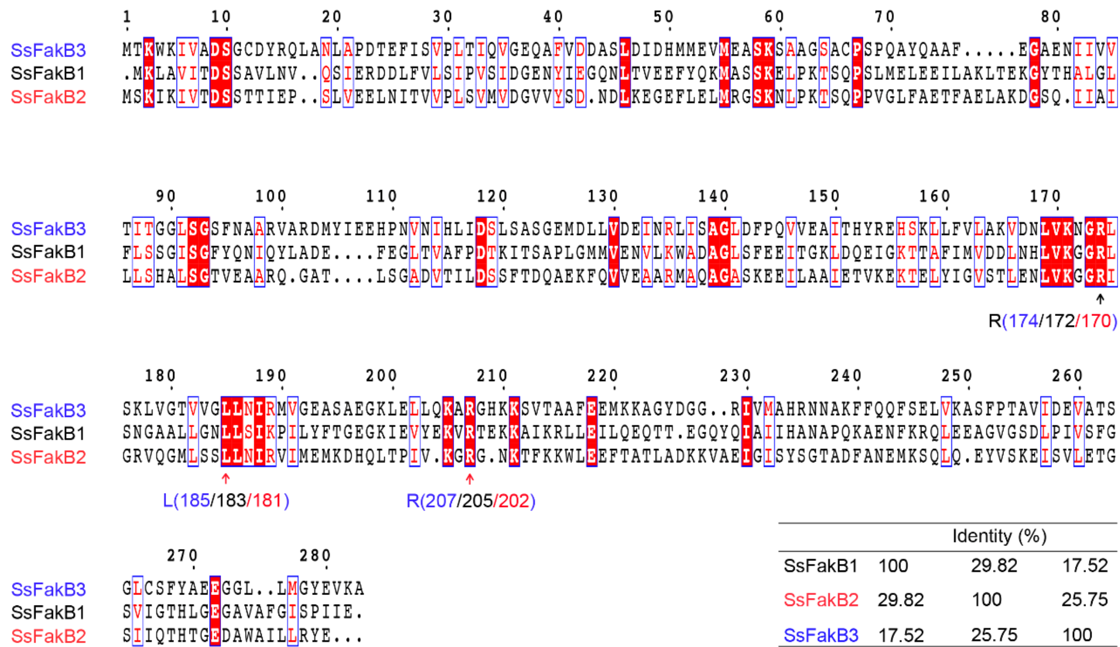


Fig. S4 Similarity analysis of FakB2 homologs amongst *S. aureus*, *S. pneumoniae* and *S. suis* 2

Sequence alignment of FakB2 was carried out as described in **Fig. S1**. The identities are listed in the inside table. The two conserved residues of FakB2 predicted to bind FakA denote leucine (L) and arginine (R). Namely, they include L181 & R202 for *S. aureus*; L182 plus R203 for *S. pneumoniae*; and L181 & R202 for *S. suis* 2. The amino acid of FakB2 that stabilizes the carboxyl group of oleate ligand corresponds to i) R170 for both SaFakB2 and SsFakB2; and ii) R171 for SpFakB2.

Abbreviations: Sa, *Staphylococcus aureus*; Sp, *Streptococcus pneumoniae*; Ss, *Streptococcus suis*



	Identity (%)		
SsFakB1	100	29.82	17.52
SsFakB2	29.82	100	25.75
SsFakB3	17.52	25.75	100

Fig. S5 Sequence comparison of three fatty acid-binding partners (FakB1, FakB2, and FakB3) from the zoonotic pathogen *S. suis* 2

Multiple sequence alignment was conducted as described in **Fig. S2**. The identities are provided in the inside table. The two critical residues having a role in binding FakA refer to leucine (L183 in SsFakB1, L181 in SsFakB2, and L185 in SsFakB3) and arginine (R205 in SsFakB1, R202 in SsFakB2, and R207 in SsFakB3). The fatty acid-binding residue denotes arginine, namely R172 for SsFakB1, R170 for SsFakB2, and R174 for SsFakB3.

Designations: Ss, *Streptococcus suis*

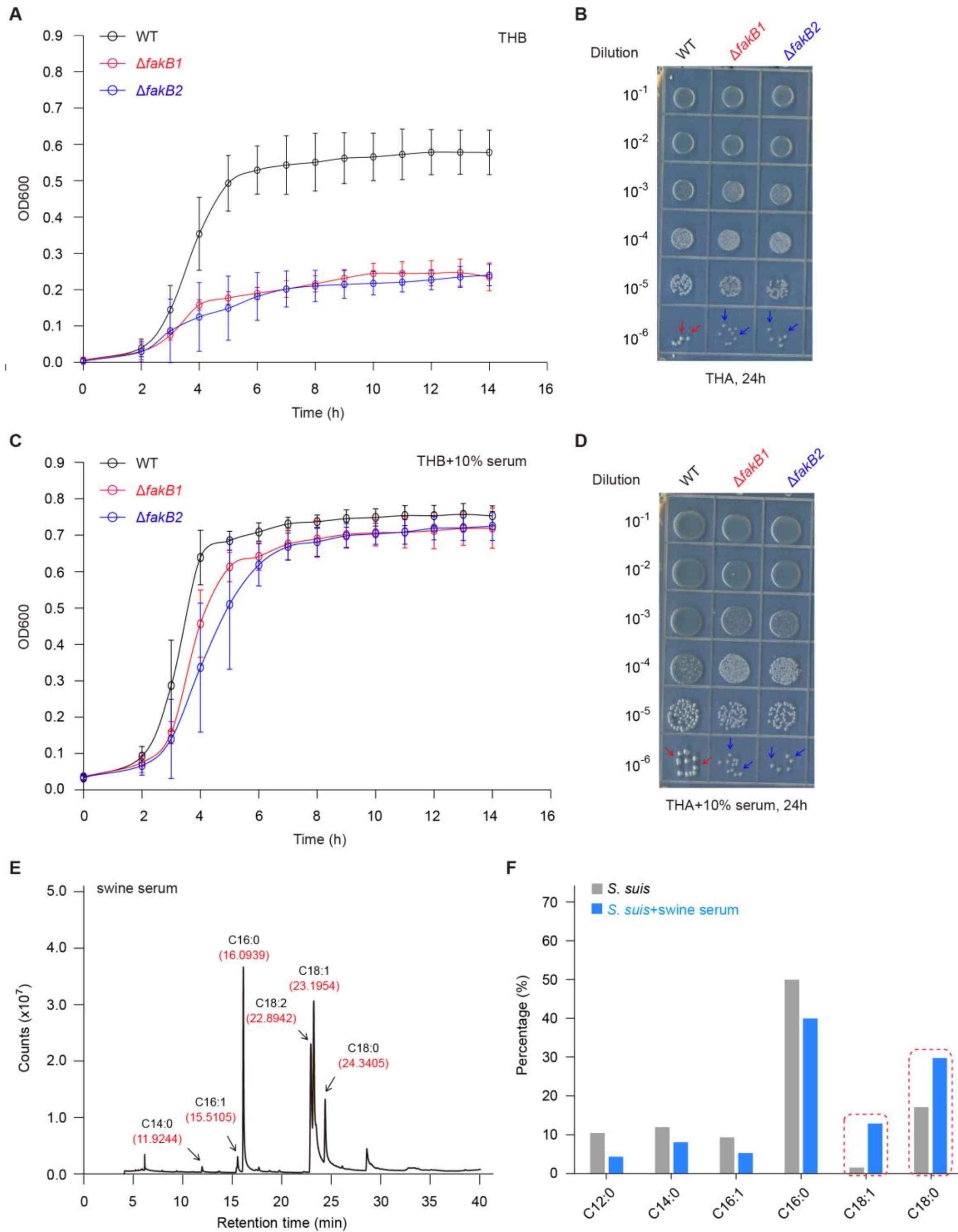


Fig. S6 Dependence of fatty acid-binding protein FakB1 (and/or FakB2) in the acquisition of serum FAs to facilitate the streptococcal growth

A. Removal of *fakB1* (or *fakB2*) attenuates the growth of *S. suis* 2 in liquid THB medium

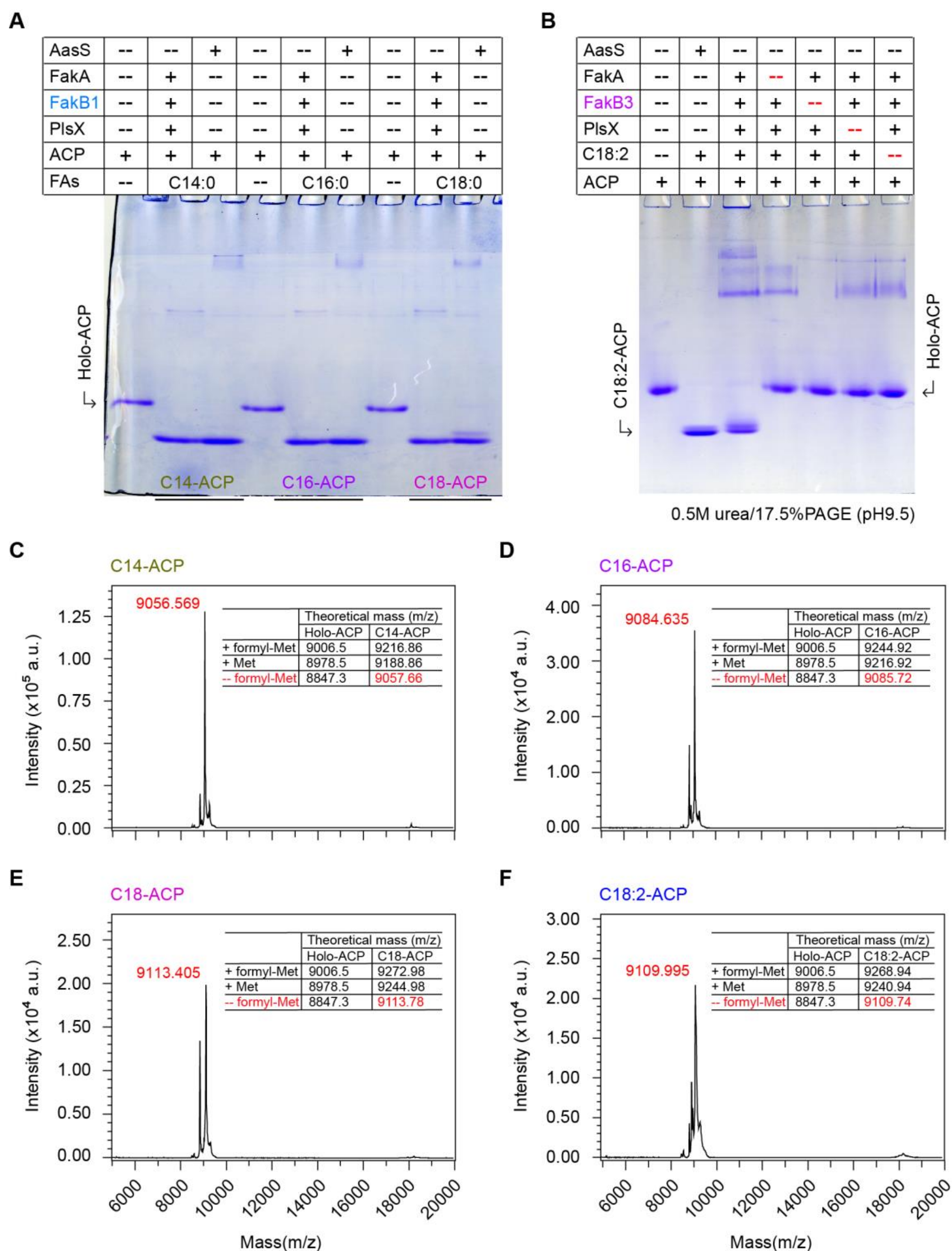
B. Compared with the wild-type, the two single mutants Δ*fakB1* and Δ*fakB2* exhibit smaller colonies on THB agar (THA) plates

Supplementation of swine serum benefits the growth of *S. suis* 2 in liquid THB medium (**C**) and on solid THA plate (**D**)

E. Gas chromatography (GC)-based fatty acid profile of swine serum

F. GC analyses of bacterial fatty acid composition suggested that the two FAs (steric acid and oleic acid) incorporated into membrane lipid are significantly elevated upon the growth of *S. suis* in the presence of swine serum

Designations: C14:0, Myristic acid; C16:0, Palmitic acid; C16:1, Palmitoleic acid; C18:0, Steric acid; C18:1, Oleic acid; C18:2, Linoleic acid.



	Holo-ACP	C14-ACP
+ formyl-Met	9006.5	9216.86
+ Met	8978.5	9188.86
-- formyl-Met	8847.3	9057.66

	Holo-ACP	C16-ACP
+ formyl-Met	9006.5	9244.92
+ Met	8978.5	9216.92
-- formyl-Met	8847.3	9085.72

	Holo-ACP	C18-ACP
+ formyl-Met	9006.5	9272.98
+ Met	8978.5	9244.98
-- formyl-Met	8847.3	9113.78

	Holo-ACP	C18:2-ACP
+ formyl-Met	9006.5	9268.94
+ Met	8978.5	9240.94
-- formyl-Met	8847.3	9109.74

Fig. S7 Enzymatic analyses for the two sets of Fak systems (FakA-FakB1 and FakA-FakB3)

A. The FakB1-including Fak system recognizes the three saturated FAs below (C14:0, C16:0, and C18:0)

B. Activation of linoleic acid (C18:2) by the Fak system depends on the presence of FakB3

The resultant acyl-ACP products are distinguished from the ACP substrate using conformationally-sensitive 17.5% PAGE (pH9.5) containing 0.5M urea.

The plus “+” denotes addition of protein (and/or FA), whereas the “--” symbol refers to no addition of protein (and/or FA).

MALDI TOF analyses for C14-ACP (**C**), C16-ACP (**D**) and C18-ACP (**E**), three kinds of products from the FakB1-centering Fak-PlsX reactions

F. MALDI TOF identification of C18:2-ACP produced by the FakB3-containing Fak-PlsX system

Designations: FA, fatty acid; ACP, Acyl carrier protein; C14:0, Myristic acid; C16:0 Palmitic acid; C18:0, Steric acid; C18:2, Linoleic acid; C14-ACP, Myristoyl-ACP; C16-ACP, Palmitoyl-ACP; C18-ACP, Stearyl-ACP; C18:2-ACP, Linoleyl-ACP.

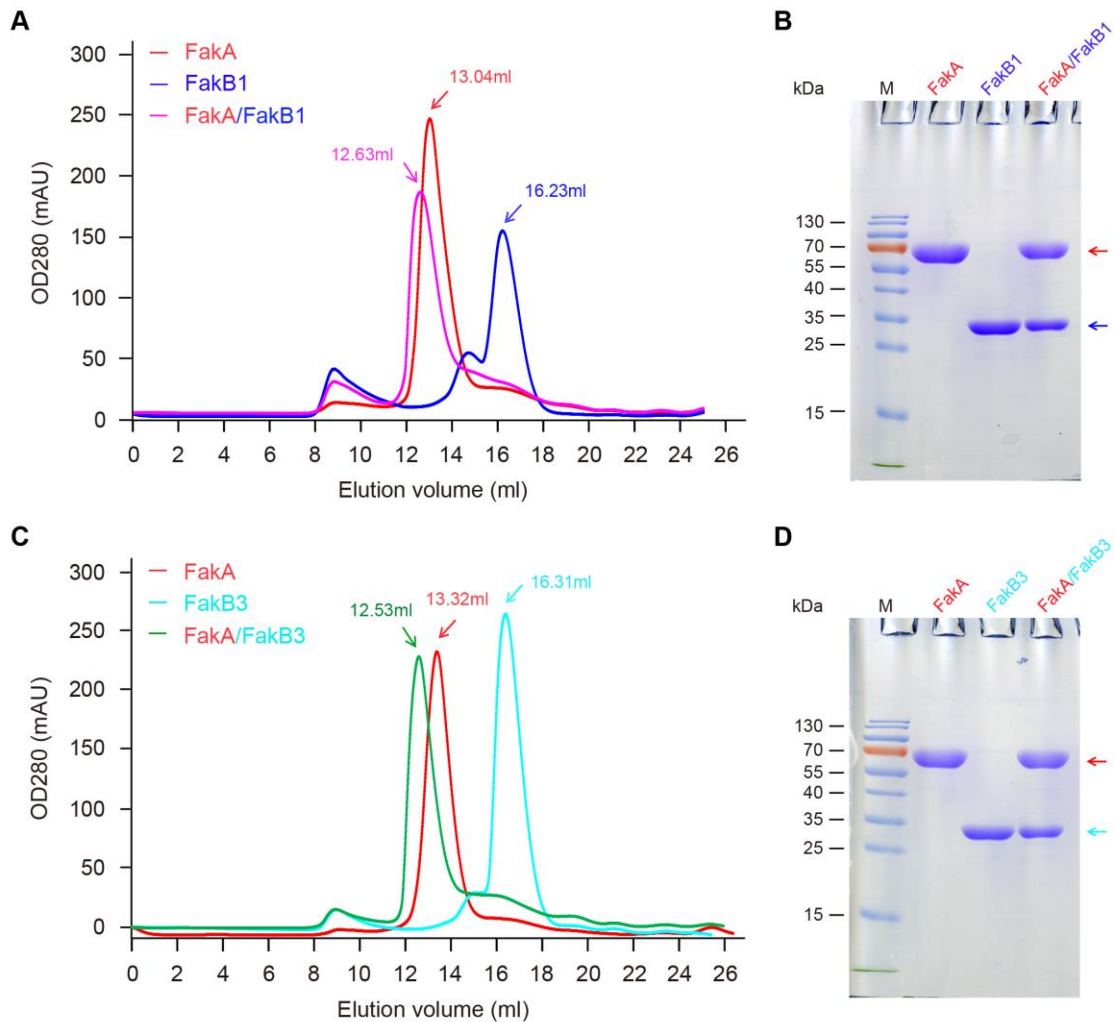


Fig. S8 Biochemical evidence for FakA binding to FakB1 and/or FakB3

A. Gel filtration analysis illustrated that the mixture of FakA/FakB1 forms a single peak and migrates faster than that of FakA (and/or FakB1) alone
B. The profile of SDS-PAGE separation revealed co-occurrence of FakA and FakB1 in a single peak of the aforementioned mixture (panel **A**)
C. A single peak formed by FakA complexed with FakB3 in gel filtration
D. Two unique proteins (FakA and FakB3) are detected by SDS-PAGE in the complex protein peak (panel **C**)
Gel filtration was conducted with a Superdex 200 increase column. Consistent with the fact that FakA binds FakB2, this demonstrated that FakA efficiently interacts with FakB1 and FakB3.

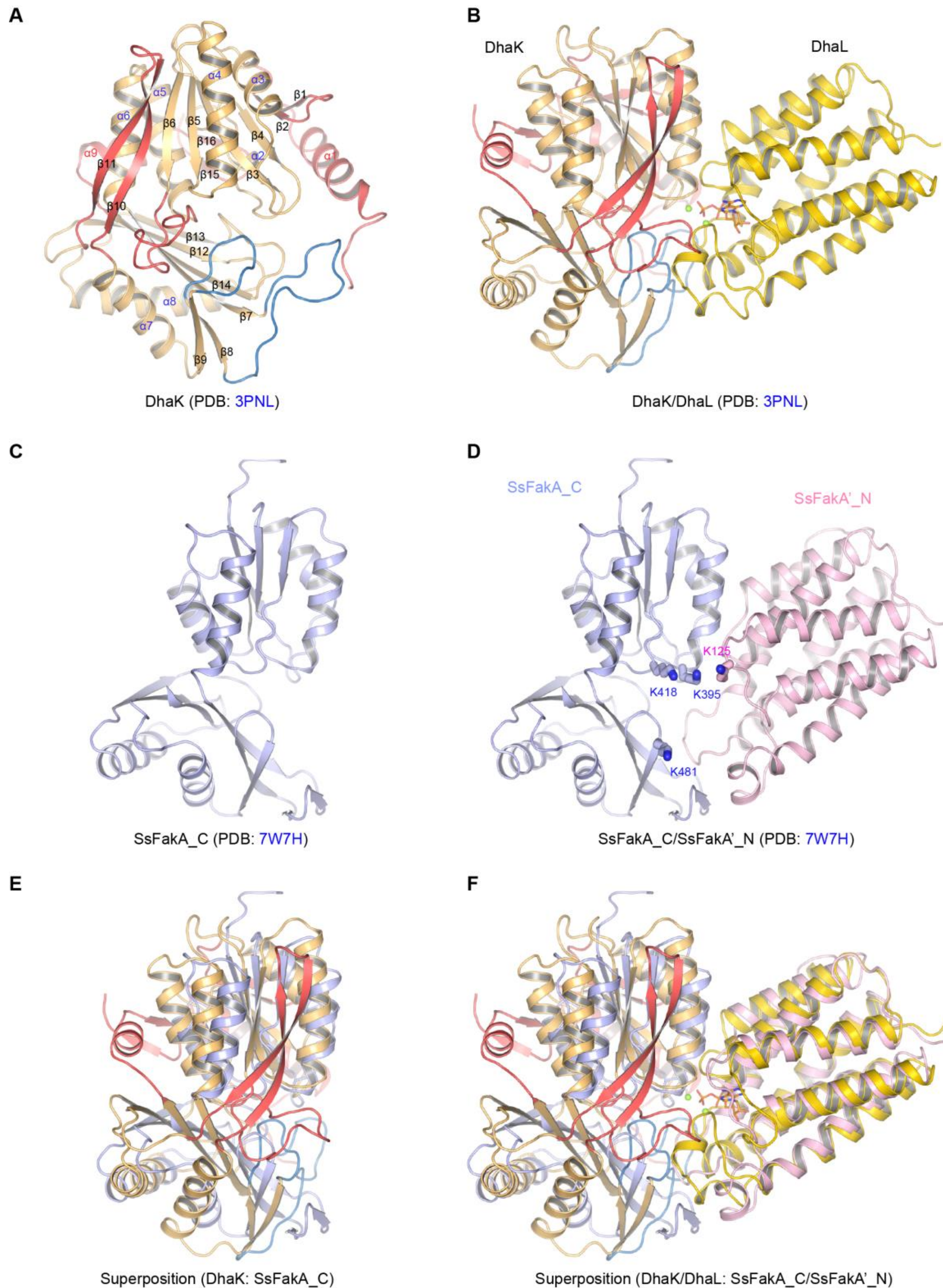


Fig. S9 Structural evidence that the *S. suis* FakA_C/FakA'_N heterodimer is analogous to the *E. coli* DhaK-DhaL kinase complex

A. Ribbon structure of the DhaK subunit of *E. coli* dihydroxyacetone (DHA) kinase system

B. Crystal structure of DhaK-DhaL complex of *E. coli*

The *E. coli* DhaK-DhaL complex defines a phosphoenolpyruvate (PEP)-dependent DHA kinase system.
C. Ribbon diagram for SsFakA_C structure
D. A heterodimer is formed by two domains of SsFakA (SsFakA_C and SsFakA'_N)
Four basic residues of lysine that surround the substrate cavity are labeled, namely K125, K395, K418, and K481. Except K125 that arises from FakA'_N domain, the remaining 3 residues originate from FakA_C domain.
E. Structural superposition of the SsFakA_C domain with *E. coli* DhaK subunit
F. Structural alignment of *S. suis* FakA_C/FakA'_N heterodimer with *E. coli* DhaK-DhaL kinase complex

Structural comparison of protein folding patterns unveiled that the gram-positive *Streptococcus* FakA is evolutionarily analogous to the gram-negative *Escherichia* DhaK/L/M kinase system.

Here, DhaK is in beige, and DhaL is shown in gold. Among them, the regions absent in SsFakA_C are colored red (extra-insertions), whereas the parts present in SsFakA_C but in different conformation are colored blue.

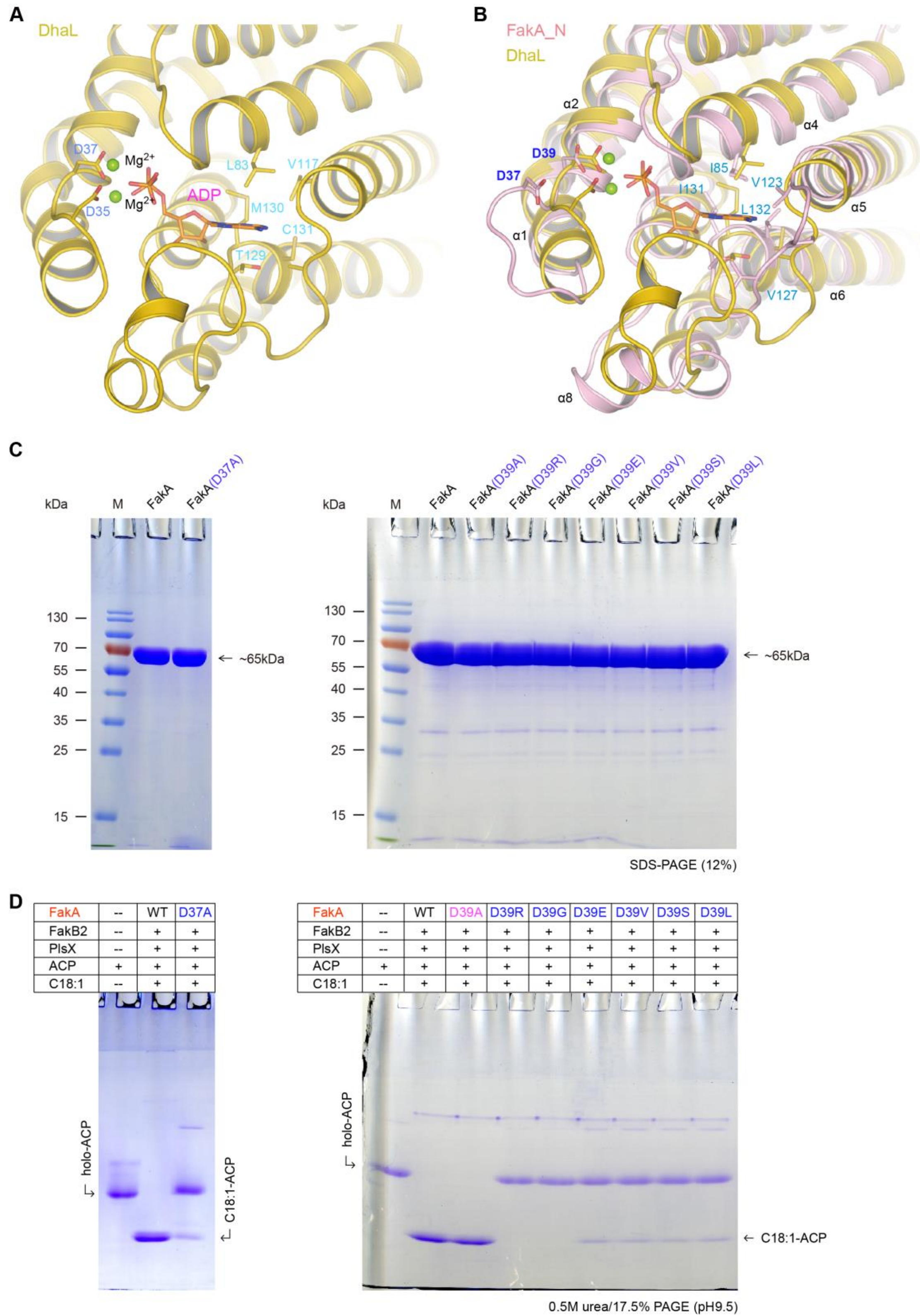


Fig. S10 Structure-guided functional analysis for FakA_N

A. Ribbon structure of DhaL (PDB: 3PNL)

The two residues (D35 and D37) of DhaL are believed to coordinate magnesium ions (green spheres). Five residues of DhaL (L83, V117, M130, C131 and T129) participate in binding the adenine moiety of ADP.

B. Structural alignment of DhaL and FakA_N

Compared with the two residues (D35 and D37) of DhaL, the counterparts of FakA_N refer to D37 and D39. Accordingly, the five ADP-interacting residues of FakA_N correspond to I85, V123, L132, V127, and I131.

C. SDS-PAGE profile of the eight FakA mutants

In addition to FakA(D37A), the remaining 7 mutants of FakA include FakA(D39A), FakA(D39R), FakA(D39G), FakA(D39E), FakA(D39V), FakA(D39S), and FakA(D39L).

D. Functional impairment of certain FakA mutants defective in coordination of magnesium

The mutation of FakA(D37A) diminished its catalytic activity. Except with FakA(D39A), the remaining six mutants of FakA are functionally impaired, namely FakA(D39R), FakA(D39G), FakA(D39E), FakA(D39V), FakA(D39S), and FakA(D39L). This indicated that the two amino acids (D37 and D39) of FakA could act as magnesium-coordinating residues, having critical roles in the Fak system.

Conformationally-sensitive gel of 0.5M urea/17.5% PAGE (pH9.5) was applied to capture the product of C18:1-ACP arising from ACP substrate in the *in vitro* reconstituted Fak system.

The plus “+” denotes addition of protein (and/or C18:1), whereas the “--” symbol refers to no addition.

Designations: M, protein marker; ACP, Acyl carrier protein; C18:1, Oleic acid; C18:1-ACP, Oleyl-ACP.

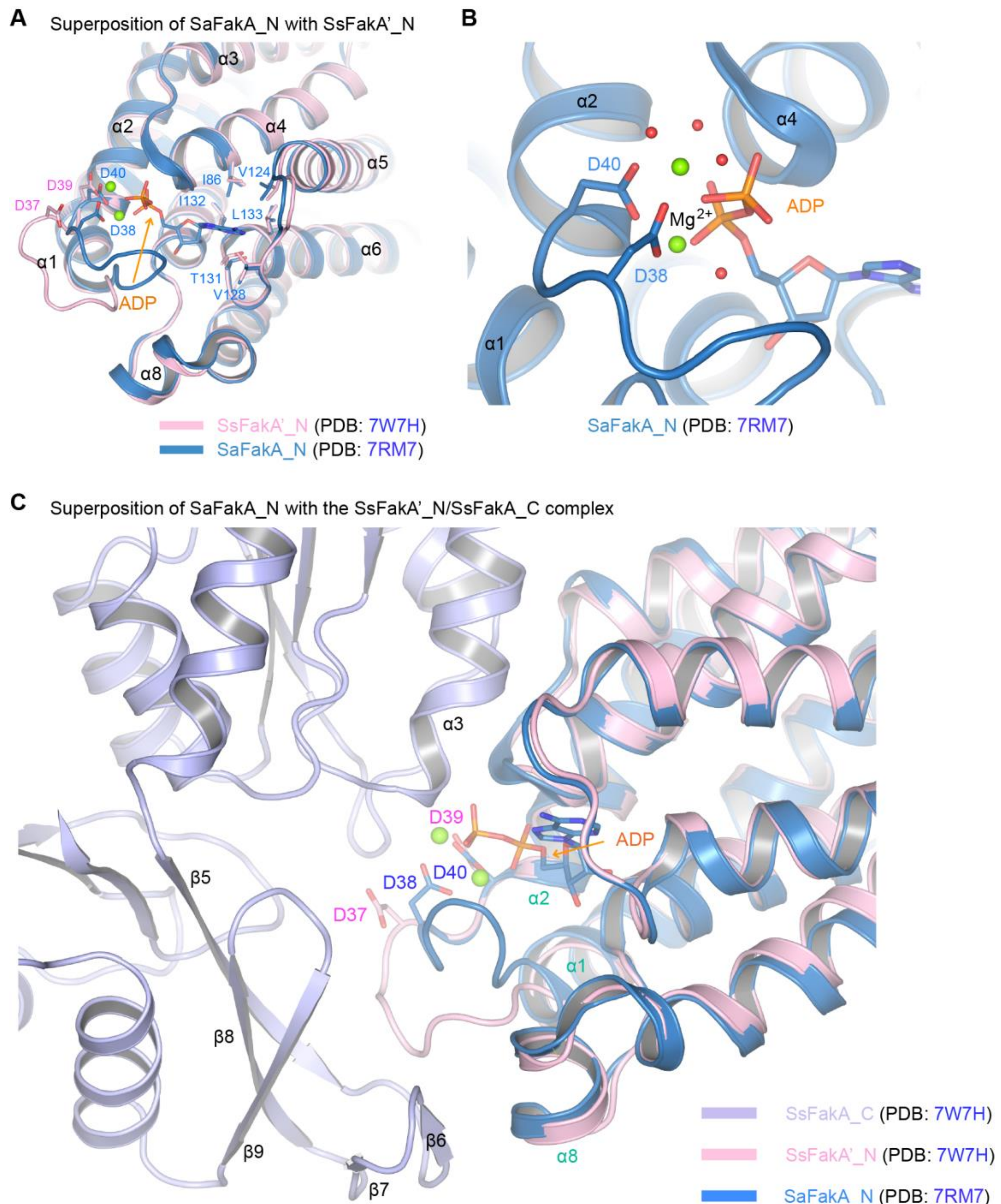


Fig. S11 Structural comparison of FakA_N domain in *S. suis* with the counterpart in *S. aureus*

A. Structural superposition of SsFakA_N from SsFakA-B2 complex with SaFakA_N-Mg^{2+}-ADP

The RMSD between the two structures is around 0.95 Å over 198 aligned Cα atoms.
Ribbon structures of SsFakA_N and SaFakA_N (PDB: 7RM7) are separately colored pink and blue. The two Mg^{2+}-coordinating residues (D38 and D40) of SaFakA_N are equivalent to D37 and D39 of SsFakA_N. The ADP-interacting

residues of SaFakA_N include I86, V124, L133, V128, T131, and I132. SsFakA_N has an identical set of residues (I85, V123, L132, V127, T130, and I131).

B. Close-up view of SaFakA_N residues coordinating Mg^{2+}-ADP
Mg^{2+} atoms are shown as green spheres, water molecules are shown as red spheres, and ADP is displayed as sticks. D38 and D40 of SaFakA_N are counterparts of D37 and D39 in SsFakA.

C. Superposition of SaFakA_N-Mg^{2+}-ADP structure with SsFakA'_N/FakA_C complex

After Mg^{2+}-ADP binding, the loop between $\alpha 1$ and $\alpha 2$ shifted upward compared to the ADP-free structure.

Designations: Ss, *Streptococcus suis* (*S. suis*); Sa, *Staphylococcus aureus*

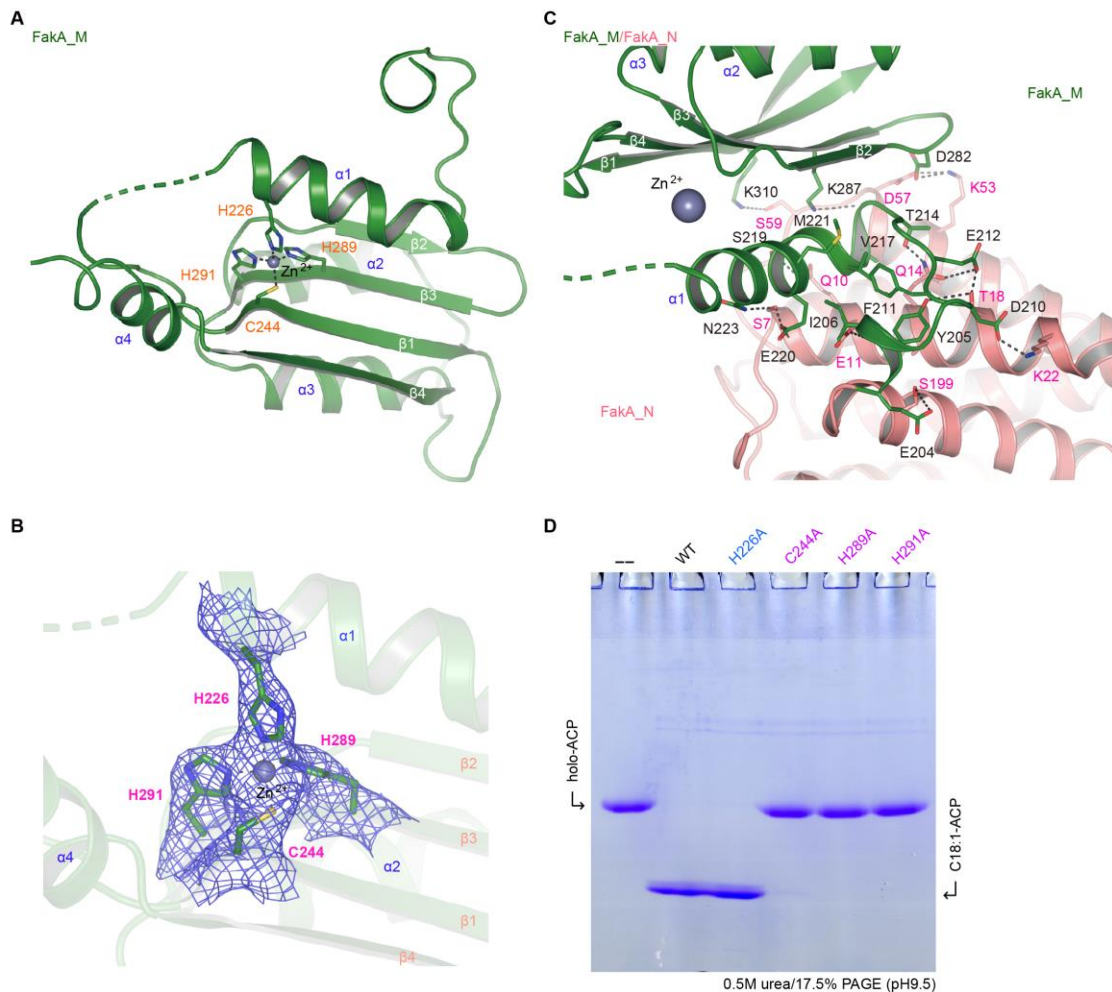


Fig. S12 FakA_M zinc finger motif and FakA_M-FakA_N interaction

A. Close-up view of the zinc finger motif in FakA_M
Zinc atom (grey sphere) is coordinated by H226, C244, H289, and H291.

B. A simulated-annealing 2Fo-Fc composite omit map contoured at 2.0σ for the Zinc finger residues

C. Interface residues between FakA_M and FakA_N
Four residues of FakA_M (M221, V217, F211, and I206) form a small hydrophobic core and stabilize α1-helix and a loop in front of this helix. Residues from the α1-helix and its adjacent loop form an extensive hydrogen bonding network with FakA_N. The α1-helix is linked to the β-sheet through the coordinated zinc. A few residues (e.g., D282, K287, and K310) from this β-sheet also contribute to the FakA_M interaction with FakA_N. Dashed lines denote hydrogen bonds and salt bridges.

D. Functional analyses of the four residues (H226, C244, H289, and H291) forming the zinc finger motif

Except H226A, the other three mutants of FakA (C244A, H289A, and H291A) largely lost enzymatic activities. This underscores the critical role of the zinc finger domain in FakA kinase.

The *in vitro* reconstituted Fak-PlsX system was used to test the enzymatic activities of FakA and its four single mutants of zinc finger-forming residues. The product C18:1-ACP was separated from the ACP substrate by using the conformationally-sensitive gel of 0.5M urea/17.5% PAGE (pH9.5).

WT denotes the wild-type FakA enzyme, and the “--” symbol refers to no addition of FakA.

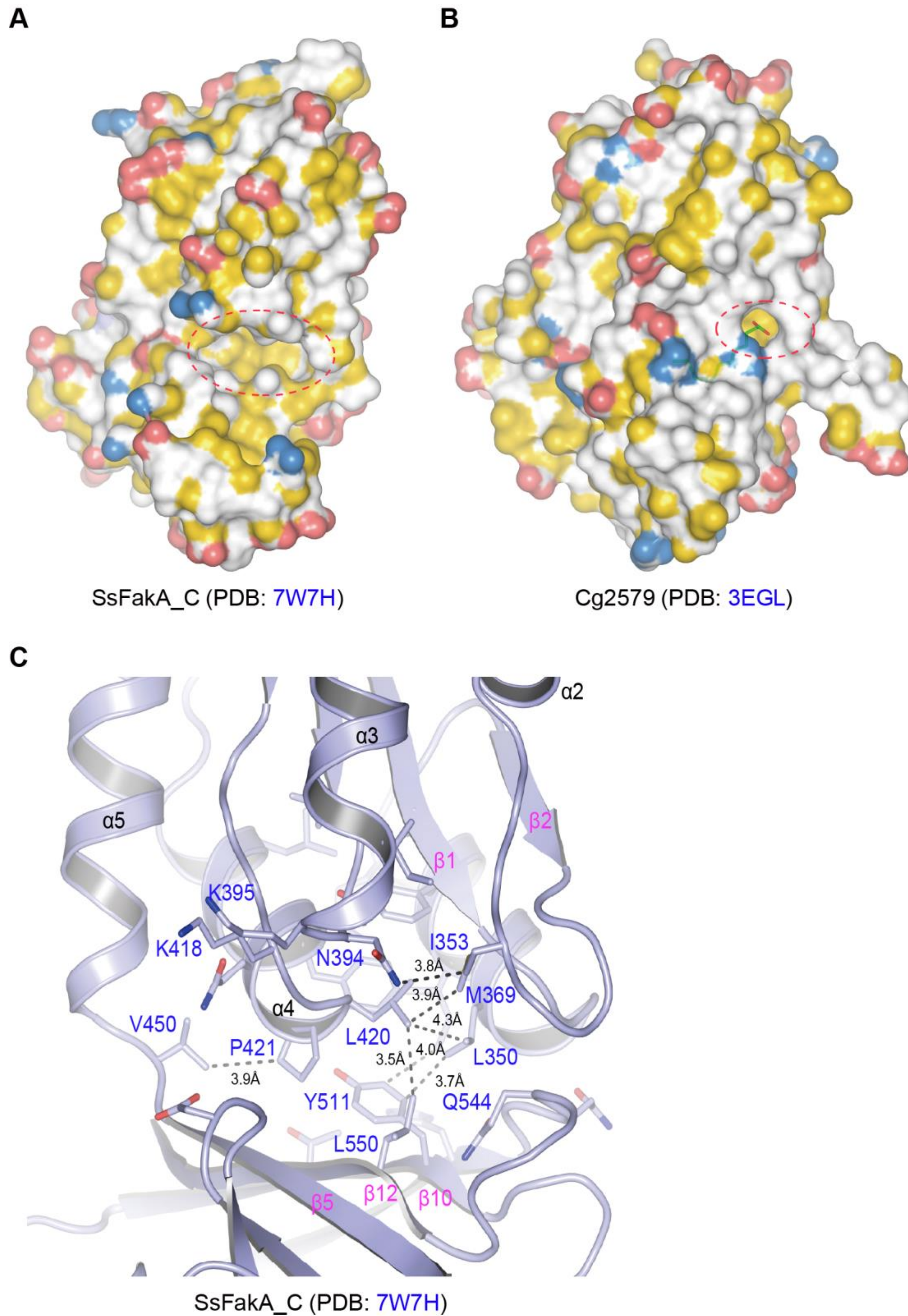


Fig. S13 FakA_C inside is different from that of DegV, the paradigm member of fatty acid-binding proteins

A. Surface representation of SsFakA_C

It is shown in the YRB color scheme. The hydrophobic, positively-charged, and

negatively-charged atoms are colored yellow, blue, and red, respectively. All the other atoms are given in white. Dashed line oval denotes the shallow wide pocket in SsFakA.

B. YRB color scheme for the structure of Cg2579

Cg2579 possesses a narrow, deep cavity harboring fatty acid.

C. Ribbon illustration for the residues inside SsFakA_C

Dashed lines indicate the distance between residues whose counterparts are involved in fatty acid binding in Cg2579, which is very small. As a result, there is no place for the fatty acids in SsFakA_C in current conformation.

Designations: Ss, *S. suis*.

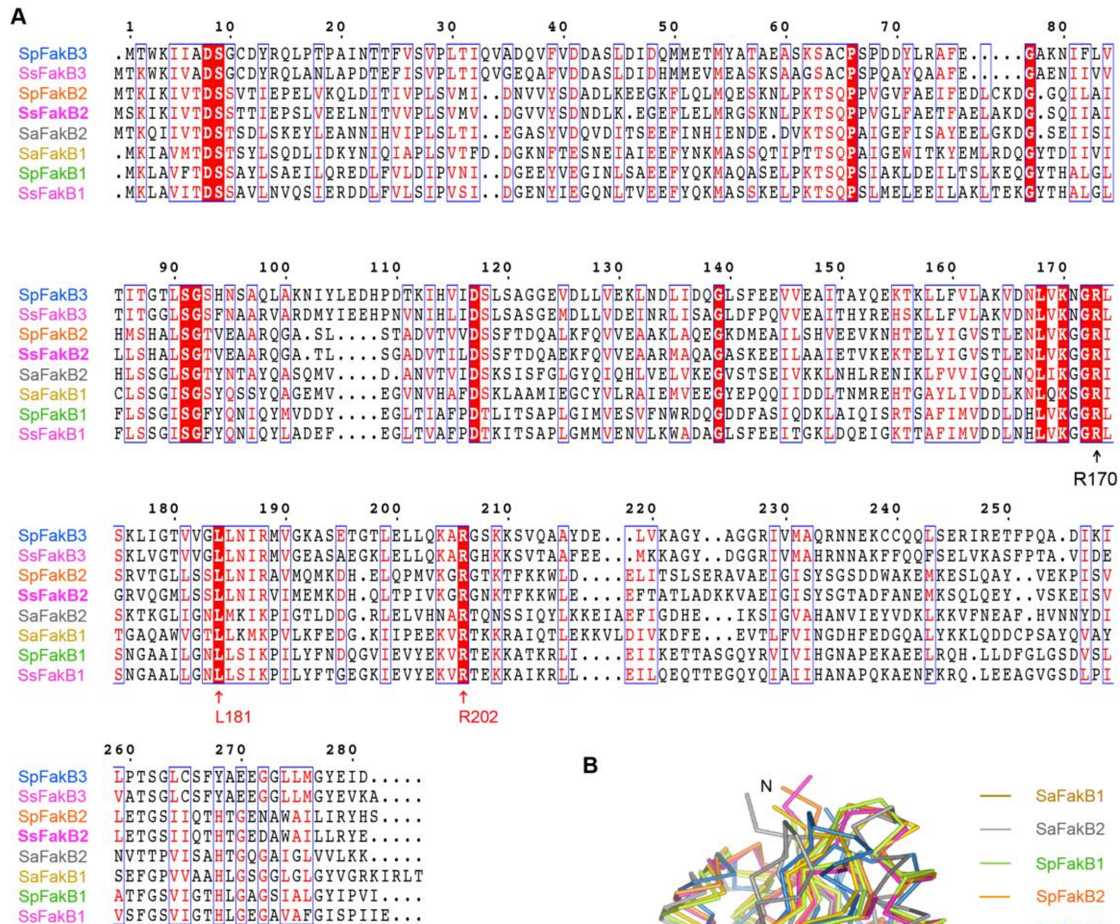


Fig. S14 Sequence and structural alignment of FakB homologs

A. Multiple sequence alignment of eight FakB homologs from different species

B. Structural alignment of six FakB proteins with known structures

The two critical residues having a role in binding FakA refer to leucine (e.g., L181 in SsFakB2) and arginine (such as R202 in SsFakB2). The fatty acid-binding residue denotes arginine, namely R170 for SsFakB2.

Designations: Sa, *Staphylococcus aureus*; Sp, *Streptococcus pneumoniae*; Ss, *Streptococcus suis*

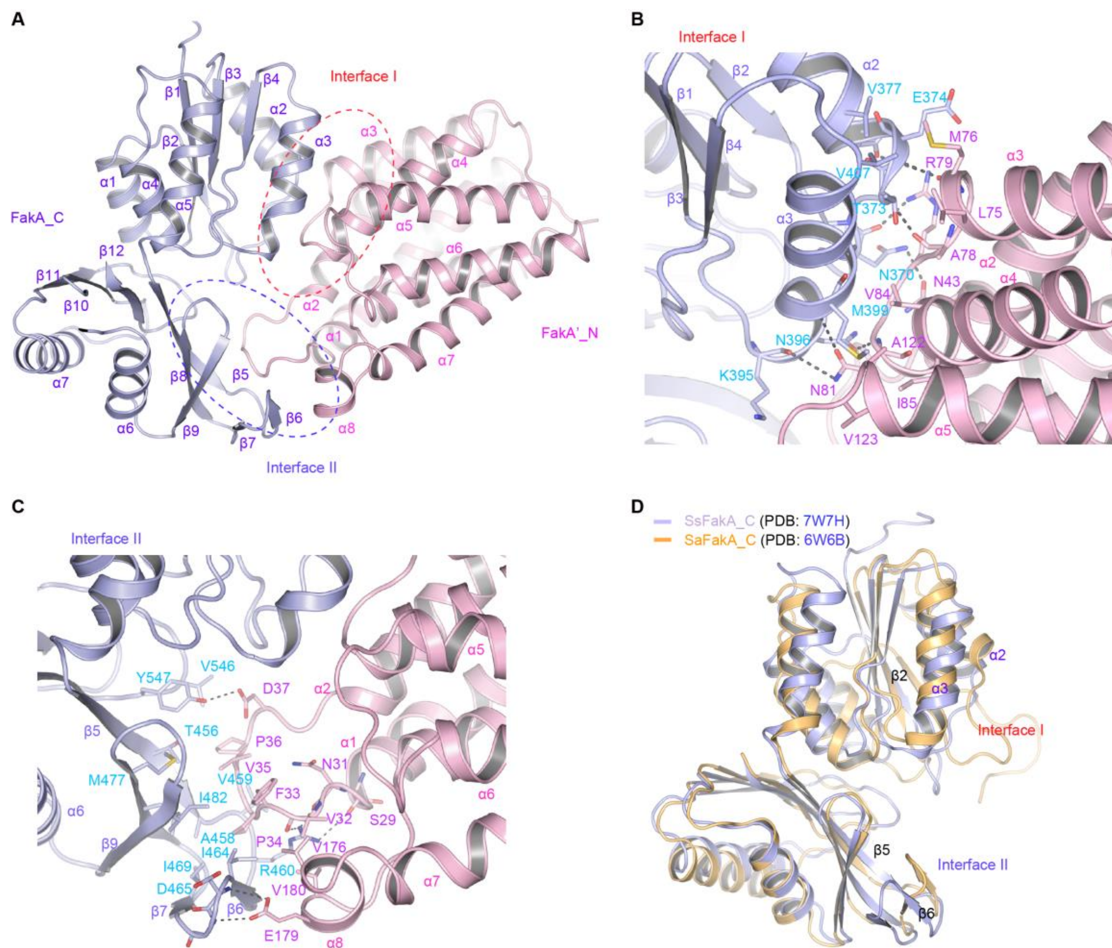


Fig. S15 Dissecting the interaction interface between FakA_C and FakA'_N

A. Structural snapshot of FakA_C bound by FakA'_N
The two interaction interfaces are highlighted by dashed line ovals.

B. Close-up view of the interface I

As for FakA'_N, N81 forms hydrogen bonds with K395 and M399 main chain atoms from FakA_C, and R79 interacts with both N370 main chain carbonyl group and D375 side chain of FakA_C. Similarly, N43 of FakA'_N and N370 of FakA_C form a hydrogen bond. In addition, two hydrogen bonds occur between A78/M76 main chain carbonyl groups and amide groups of T373/E374. M399 side chain inserts into a hydrophobic pocket formed by four FakA'_N residues (A122, V123, I85, and V84). The two amino acids (M76 and L75) located in α3-helix of FakA'_N also have hydrophobic interactions with the two residues (V377 and V407) of FakA_C. Dashed lines denote hydrogen bonds (and/or salt bridges).

C. Close-up view of Interface II

E179 of FakA'_N forms two hydrogen bonds with the mainchain amide group of D465 and G466 of FakA_C. By contrast, R460 of FakA_C forms 3 hydrogen bonds with mainchain carbonyl group of the three residues of FakA'_N (S29, N31, and V32). Similarly, Y547 from FakA_C interacts with D37 of FakA'_N via a hydrogen bond. Additionally, the following five residues of FakA_C (I464, I482, I469, L475, and M477) constitute a hydrophobic patch and interact with four amino acids (F33, V176, V35, and V32) in FakA'_N.

D. Structural superposition of SsFakA_C (PDB: 7W7H) and SaFakA_C (PDB: 6W6B)

The RMSD of the two structures is around 2.79 Å over 187 aligned Cα atoms.

Designations: Ss, *S. suis*; Sa, *S. aureus*.

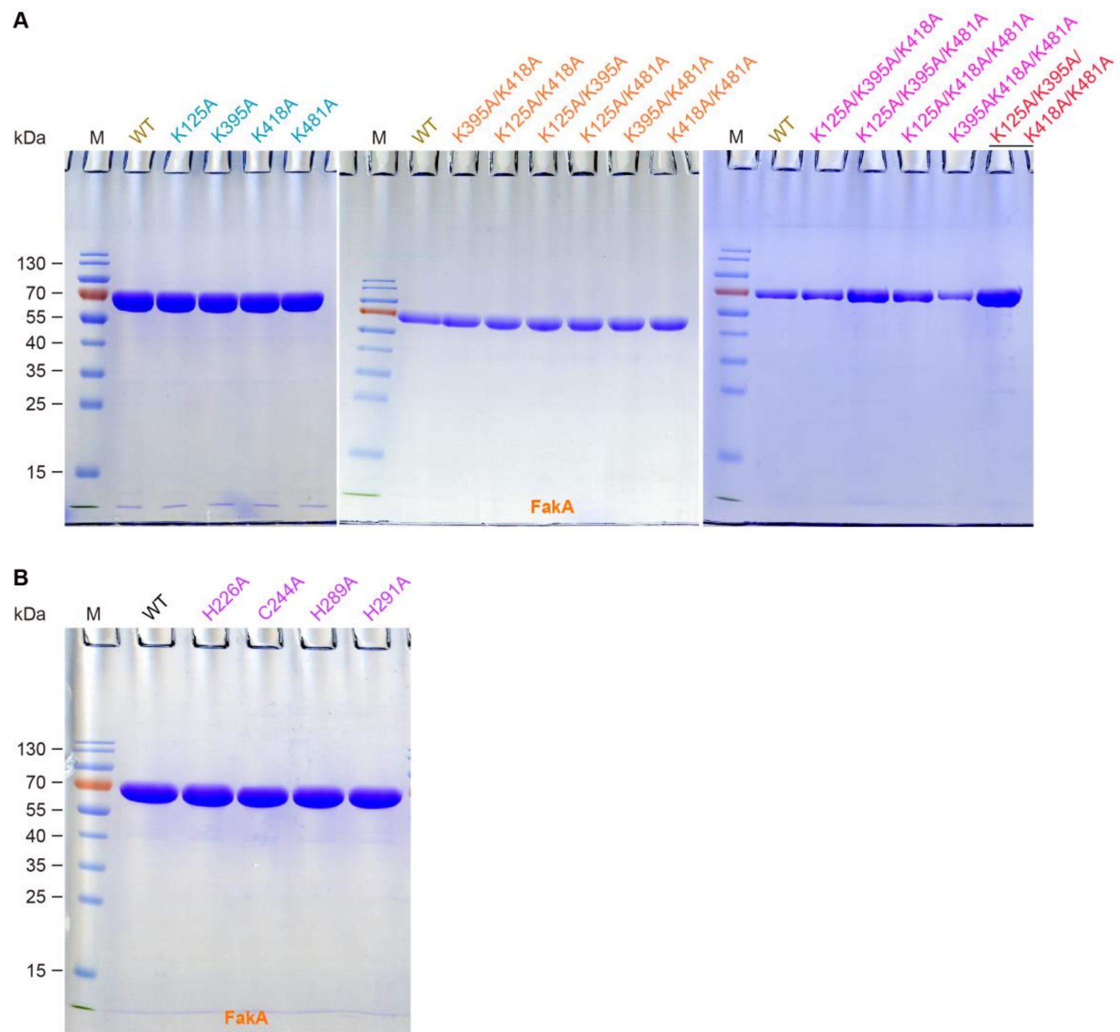


Fig. S16 SDS-PAGE profile of a panel of FakA mutants

A. SDS-PAGE analyses of the 15 FakA mutants

The 15 FakA mutants correspond to 4 single mutants, 6 double mutants, 4 triple mutants and 1 quadruple mutant.

B. Use of SDS-PAGE to verify the purity of the four single FakA mutants of which certain zinc finger-forming residue is replaced with alanine

Namely, they include H226A, C244A, H289A, and H291A.

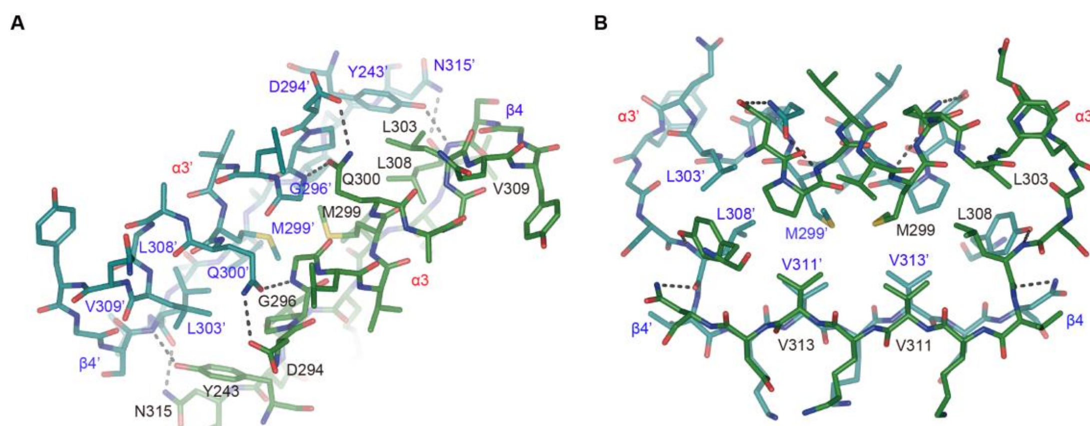


Fig. S17 Detailed analysis of FakA_M homodimer interface

A. View of FakA_M homodimer mediated by residues (shown as sticks) from $\alpha3$ and $\beta4$
The three residues (N315, Y243 and Q300) form hydrogen bonds with main chain atoms (L308' and G296') as well as side chain atoms (D294') from the other protomer.

B. Side view of $\alpha3$ and $\beta4$ packing with $\alpha3'$ and $\beta4'$, highlighting the hydrophobic residues

A group of hydrophobic residues (M299, L303, L308, V311, and V313) form an extensive hydrophobic interface between the two protomers. Dashed lines denote hydrogen bonds.

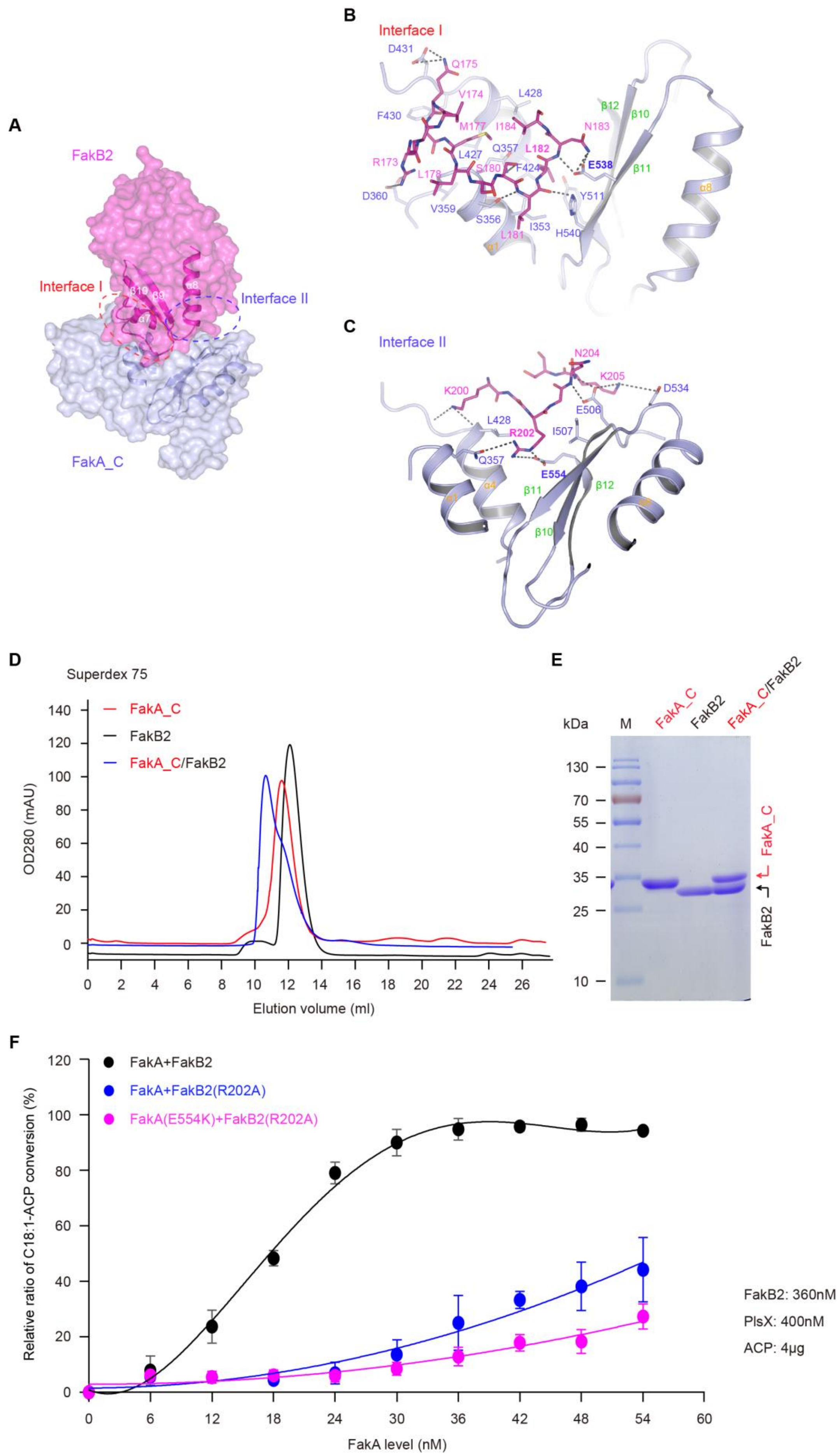

A
FakB2
Interface I
Interface II
FakA_C
B
Interface I
C
Interface II
D
Superdex 75
FakA_C
FakB2
FakA_C/FakB2
OD280 (mAU)
Elution volume (ml)
E
kDa
M
FakA_C
FakB2
FakA_C/FakB2
130
70
55
40
35
25
10
F
FakA+FakB2
FakA+FakB2(R202A)
FakA(E554K)+FakB2(R202A)
Relative ratio of C18:1-ACP conversion (%)
FakA level (nM)
FakB2: 360nM
PlsX: 400nM
ACP: 4μg

Fig. S18 Detailed analysis of FakA_C and FakB2 interaction interface

A. Overall view of FakA_C and FakB2 interaction

The two partners (FakB2 and FakA_C) are displayed with a semi-transparent surface. Ribbon diagram is given to highlight the two potential interaction interfaces (designated as Interface I and Interface II). Dashed line oval is separately colored red for Interface I, and blue for Interface II.

B. Close-up view of the interaction interface I

Five residues from FakA_C (E538, H540, S356, Q357, and D431) form eight hydrogen bonds with FakB2. Of note, E538 carboxyl group forms 2 hydrogen bonds with N183 side chain amide group and one hydrogen bond with the main chain amide group. D360 of FakA_C forms two salt bridges with R173 from FakB2. A group of conserved hydrophobic residues (L181, L182, M177, V174, and L178) in FakB2 interact with the hydrophobic patch formed by five residues of FakA_C (Y511, I353, F424, L427, and V359). Dashed lines denote hydrogen bonds and salt bridges.

C. Close-up view of the interaction interface II

Four residues of FakB2 (K200, R202, N204, and K205) form nine hydrogen bonds (and/or salt bridges) with FakA_C. In particular, R202 carbon side chain of FakB2 sits in a pocket of FakA_C, which is formed by I428, I507 and E554 side chains. Its guanidino group forms specific interactions with Q357 and E554.

D-E. Combined evidence of gel filtration with protein electrophoresis that FakA_C retains an ability to bind FakB2

F. Quantification of FakA (and/or E554A mutant) binding to its partner FakB2 (and/or R202A mutant)

The *in vitro* reconstituted system of FakA/FakB2-PlsX was applied to assay Fak activity, and conformationally-sensitive gel of 0.5M urea/17.5% PAGE (pH9.5) was used to detect the oleyl-ACP product. The ratio of oleyl-ACP product to its reactant ACP was measured through Image J software-aided acquisition of protein band intensity. It was given as an average ± SD (standard deviations) of three independent experiments.

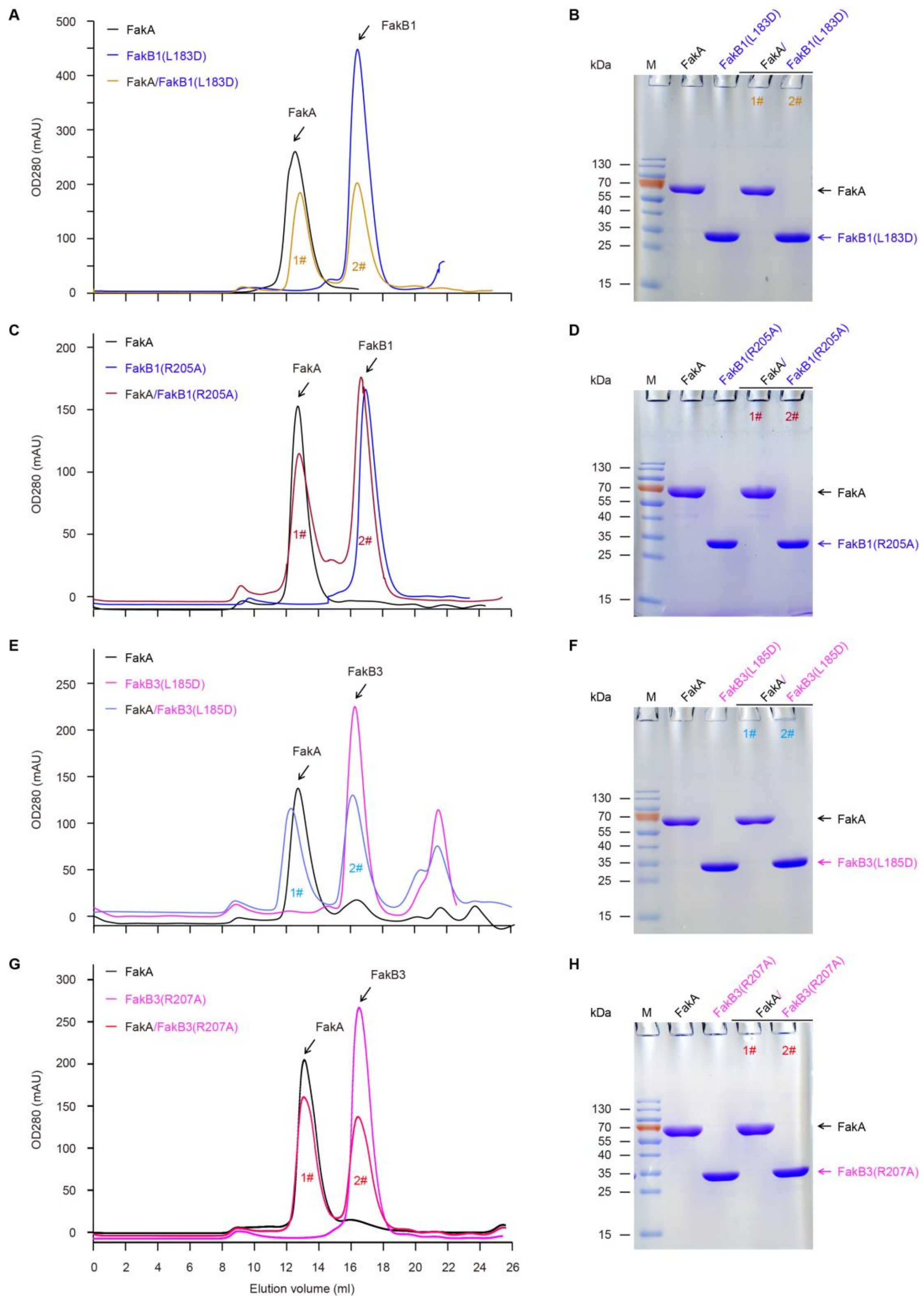


Fig. S19 Biochemical evidence that a single mutation of L183D/R205A (and/or L185D/R207A) impairs the ability of FakB1 (and/or FakB3) to bind FakA kinase

A-B. Integrative data of gel filtration with protein electrophoresis revealed the

inability of the mutant FakB1(L183D) to interact with FakA protein

C-D. The FakB1(R205A) mutant is nonfunctional in binding FakA kinase

E-F. Size exclusion chromatography profile determined that FakB3(L185D) is inactive in the interaction with FakA partner

G-H. Gel filtration confirmed that FakB3(R207A) cannot bind FakA

The mixture of FakA and FakB1(L183D or R205A) give two individual peaks (numbered 1# and 2#) following gel filtration. SDS-PAGE (12%) profile validated that FakA occurs in peak 1#, and FakB1(L183D in panels **A**-**B** or R205A in panels **C**-**D**) is eluted in peak 2#. A similar scenario was seen in the case of FakA plus either FakB3(L185D, panels **E**-**F**) or FakB3(R207A, panels **G**-**H**). The results demonstrated that none of the following four mutant proteins [namely FakB1(L183D), FakB1(R205A), FakB3(L185D), and FakB3(R207A)] can be bound by FakA enzyme. Gel filtration was conducted with a Superdex 200 increase column.

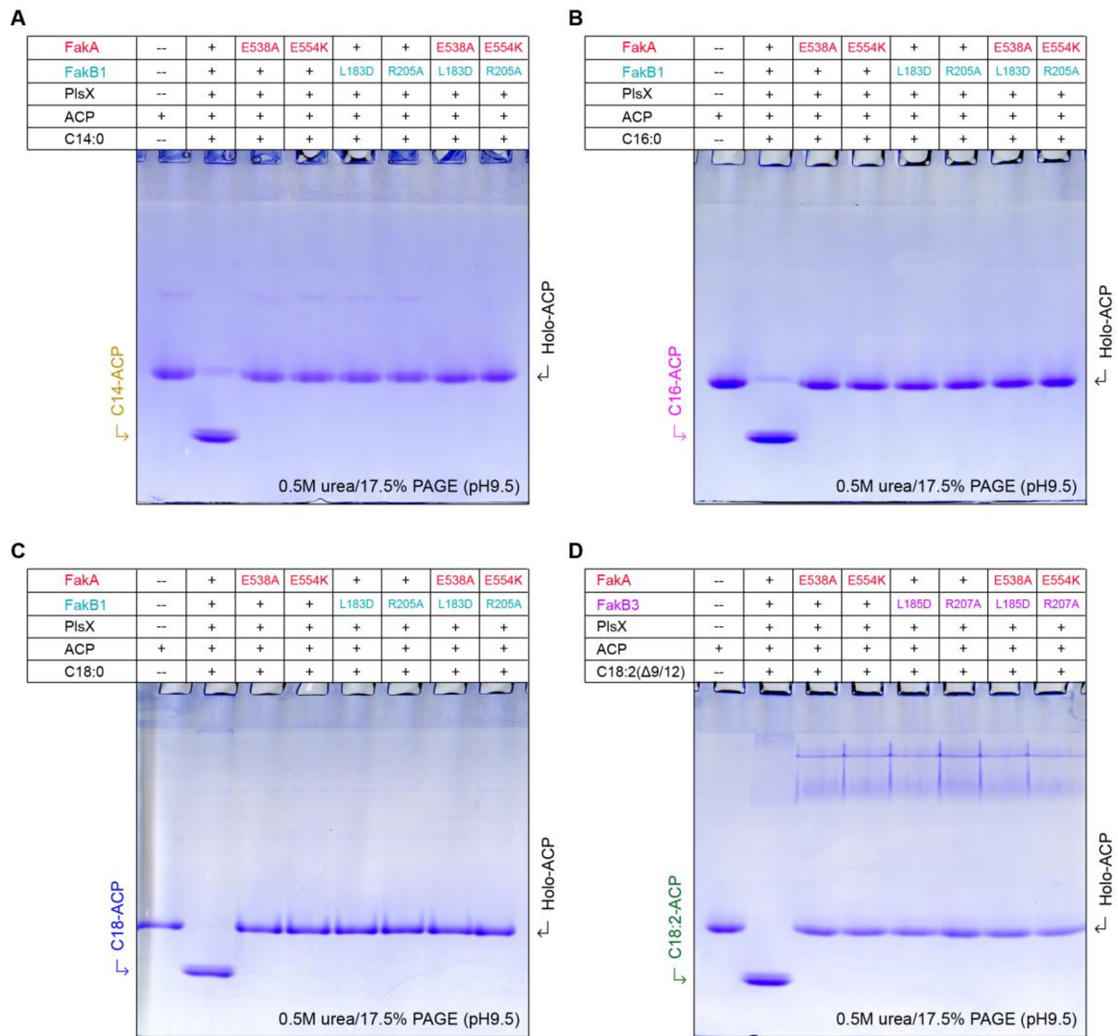


Fig. S20 Use of the *in vitro* reconstituted Fak system to probe functional roles of FakA-FakB1(FakB3) interface in the salvage of exogenous fatty acids

Impairment of FakA-FakB1 interaction disrupts its ability to utilize the three saturated fatty acids, C14:0 (**A**), C16:0 (**B**), and C18:0 (**C**)

D. Inability of FakA binding to FakB3 causes function loss of FakA-FakB3 system in phosphorylation of linoleic acid (C18:2)

Along with the two FakA residues (E538 & E554), the two sites of FakB1(L183 and R205) participate in the maintenance of FakA-FakB1 complex. Similarly, the counterparts of FakB2 correspond to L185 and R207, respectively (**Fig. S5**).

Acyl-ACP species are distinguished from the reactant ACP using the conformationally-sensitive gel of 0.5M urea/17.5% PAGE (pH9.5).

The plus (+) symbol denotes the presence of protein (and/or fatty acids), whereas the minus (--) indicates no addition.

Designations: FA, fatty acid; ACP, Acyl carrier protein; C14:0, Myristic acid; C16:0, Palmitic acid; C18:0, Steric acid; C18:2, Linoleic acid; C14-ACP, Myristoyl-ACP; C16-ACP, Palmitoyl-ACP; C18-ACP, Stearyl-ACP; C18:2-ACP, Linoleyl-ACP.

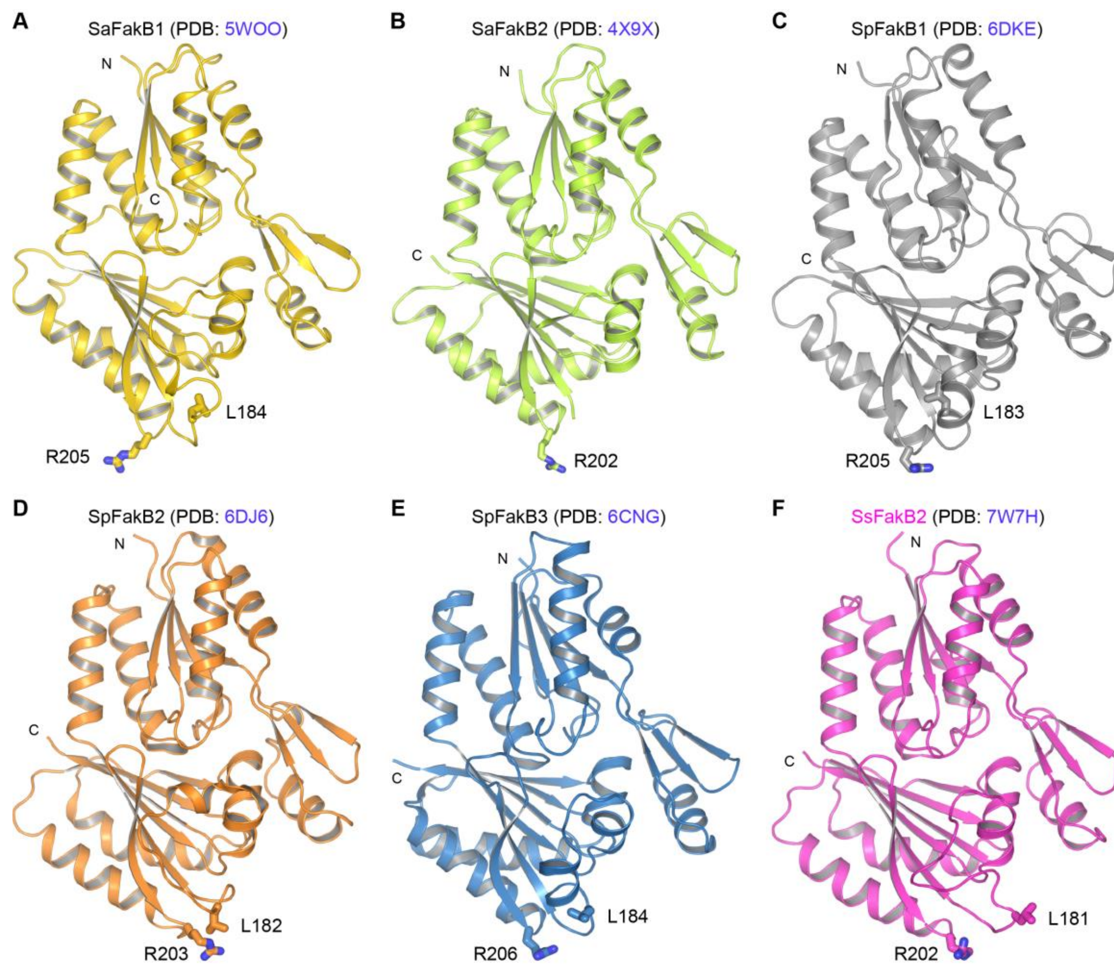


Fig. S21 Structural parallel amongst six fatty acid-binding proteins

A. Ribbon structure of SaFakB1 protein (PDB: 5WOO)
B. Ribbon presentation of SaFakB2 structure (PDB: 4X9X)
C. Structural characterization of SpFakB1 protein (PDB: 6DKE)
D. Ribbon illustration for SpFakB2 structure (PDB: 6DJ6)
E. Architecture for SpFakB3 in ribbon (PDB: 6CNG)
F. Structure of the oleate-binding protein SsFakB2 (PDB: 7W7H)

The two residues of FakB that have roles in FakA binding are labeled as follows: L184 & R205 in SaFakB1, L181 (missing) & R202 in SaFakB2, L183 & R205 in SpFakB1, L182 & R203 in SpFakB2, L184 & R206 in SpFakB3, and L181 & R202 in SsFakB2.

Designations: SaFakB1, *Staphylococcus aureus* FakB1; SaFakB2, *Staphylococcus aureus* FakB2; SpFakB1, *Streptococcus pneumoniae* FakB1; SpFakB2, *Streptococcus pneumoniae* FakB2; SpFakB3, *Streptococcus pneumoniae* FakB3; SsFakB2, *Streptococcus suis* FakB2; N, N-terminus; C, C-terminus; R, Arginine; L, Leucine.

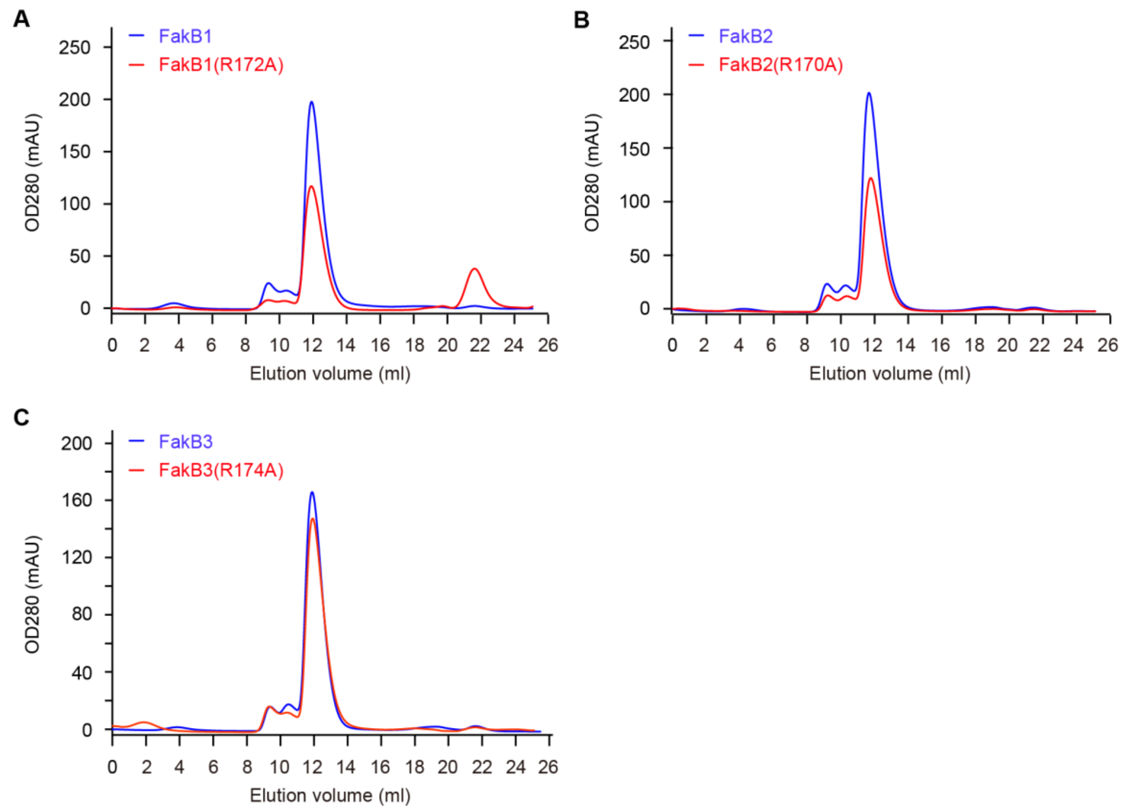


Fig. S22 Gel filtration analysis indicated that solution structures of all the three FakB (FakB1, FakB2 and FakB3) enzymes remain monomeric, regardless of the alanine substitution of the arginine residue neutralizing the ligand fatty acid carboxyl group

A. Size exclusion chromatography of the FakB1(R172A) mutant

B. Use of gel filtration to compare FakB2 and its single mutant FakB2(R170A)

C. Parallels in size exclusion chromatography profile of FakB3 and its FakB3(R174A) mutant

Size exclusion chromatography was conducted with a Superdex 75 column.

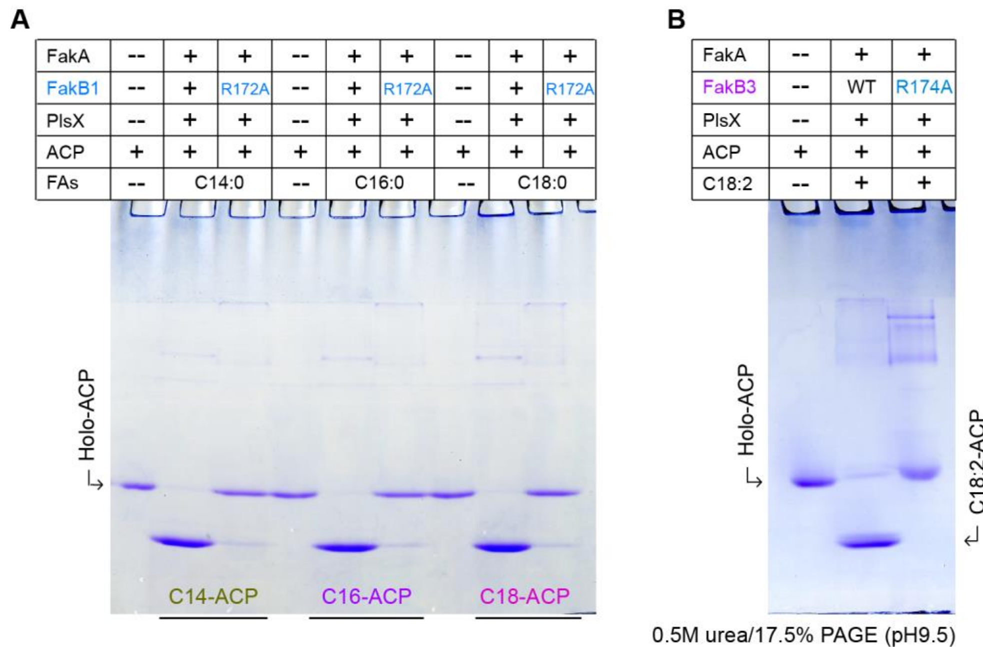


Fig. S23 Functional evaluation of the arginine residue (R172 in FakB1 and R174 in FakB3) that neutralizes extracellular FA carboxyl group

A. Enzymatic analysis revealed that the substitution of R172A impairs FakB1 binding to different saturated FA species (C14:0 to C18:0)

B. FakB3(R174A)-supplemented Fak system is inactive in scavenging linoleic acid

Acyl-ACP species are separated from the precursor by the conformationally-sensitive electrophoresis of 0.5M urea/17.5% PAGE (pH9.5).

The plus “+” denotes addition of protein (and/or FA), whereas the “--” symbol refers to no addition.

Designations: FA, fatty acid; ACP, Acyl carrier protein; C14:0, Myristic acid; C16:0 Palmitic acid; C18:0, Steric acid; C18:2, Linoleic acid; C14-ACP, Myristoyl-ACP; C16-ACP, Palmitoyl-ACP; C18-ACP, Stearyl-ACP; C18:2-ACP, Linoleyl-ACP.

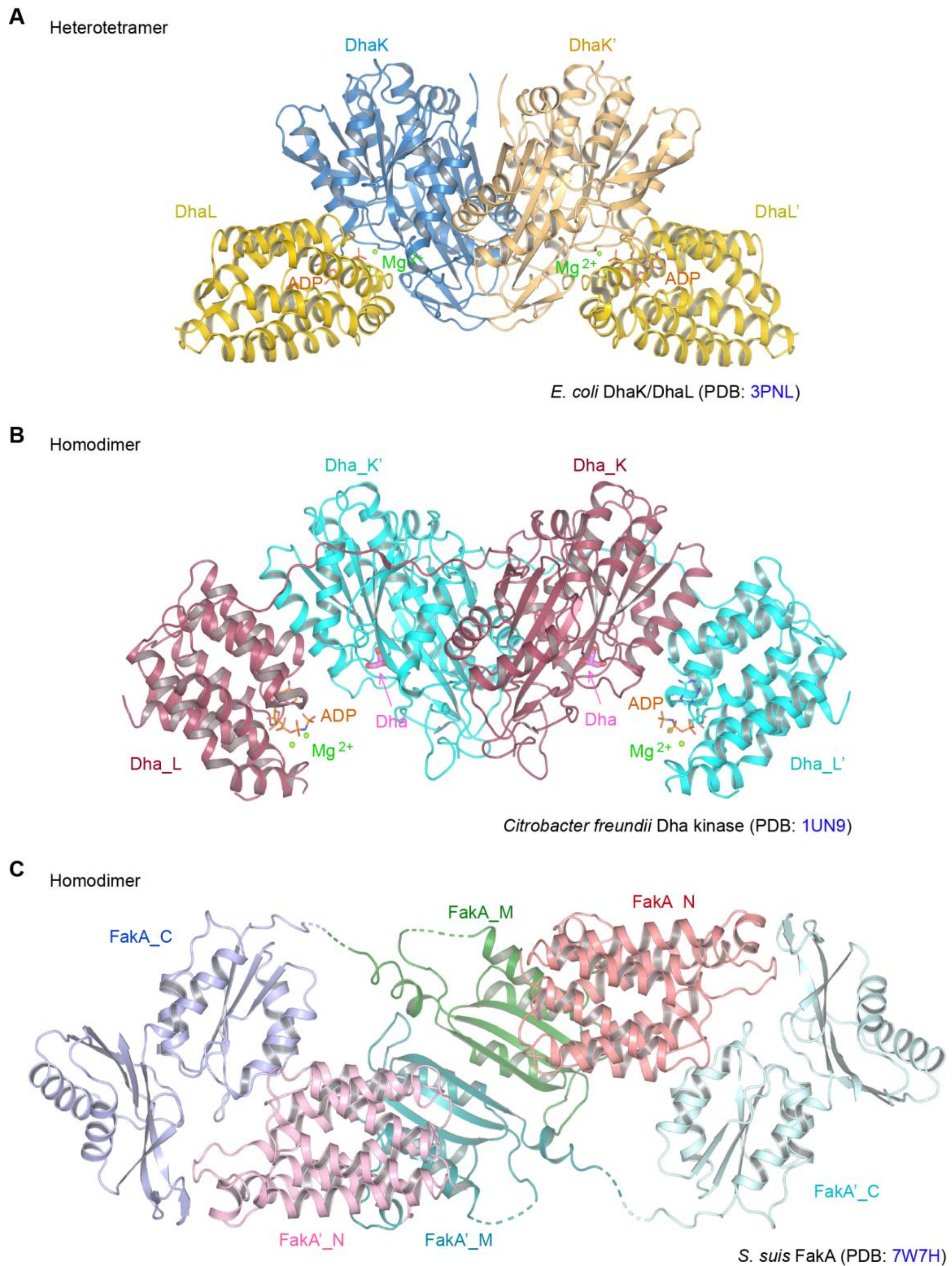


Fig. S24 Architectural comparison of *S. suis* FakA with *E. coli* DhaK/DhaL system and *Citrobacter freundii* Dha kinase

A. Ribbon structure of *E. coli* Dhak/DhaL system (PDB: 3PNL)
DhaL is colored gold, and the two DhaK molecules are separately colored blue and beige. ADP is shown with orange sticks, and Mg^{2+} is given as green spheres

B. Ribbon representation of *Citrobacter freundii* Dha kinase (PDB: 1UN9)

Dha kinase appears as a dimer, and each monomer is grouped into two subunits (Dha_K and Dha_L). The subunit of Dha_K (and/or Dha_K') is colored crimson (and/or cyan). ADP is shown with orange sticks, and Mg^{2+} is given as green spheres. Dha molecules are indicated with violet sticks.

C. Overall architecture of *S. suis* FakA dimer (PDB: 7W7H)

The three domains (FakA_N, FakA_M & FakA_C) of FakA are colored as earlier described in **Fig. 3E**.