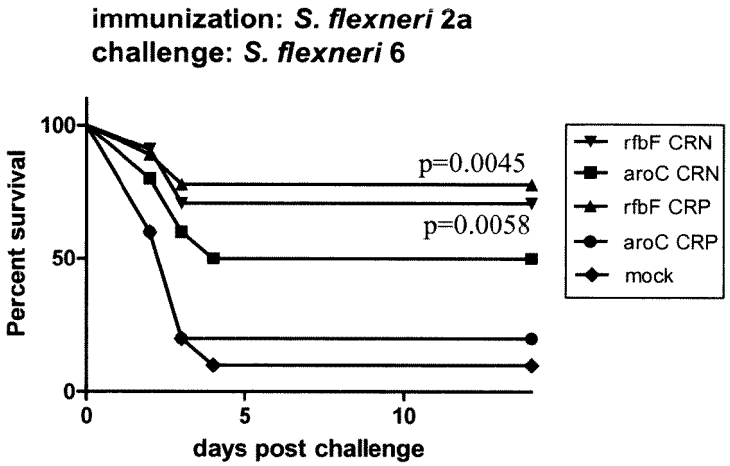
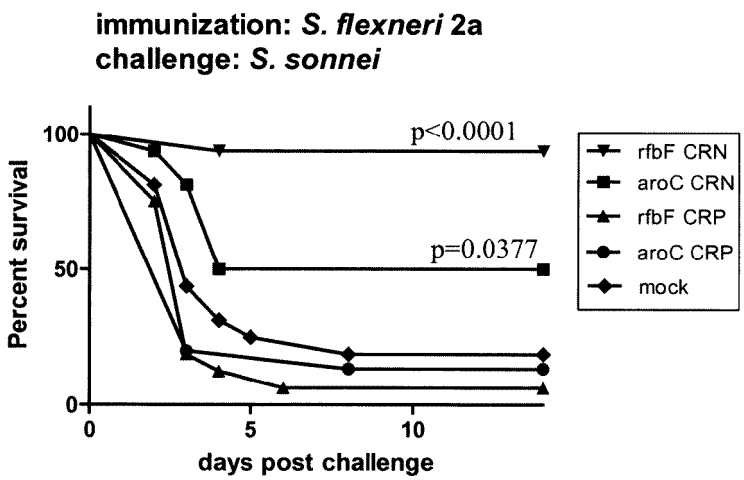
Fig. 1

a)


immunization: S. flexneri 2a
challenge: S. flexneri 6
Percent survival
100
50
0
0
5
10
days post challenge
p=0.0045
p=0.0058
rfbF CRN
aroC CRN
rfbF CRP
aroC CRP
mock

b)


immunization: S. flexneri 2a
challenge: S. sonnei
Percent survival
100
50
0
0
5
10
days post challenge
p<0.0001
p=0.0377
rfbF CRN
aroC CRN
rfbF CRP
aroC CRP
mock

c)

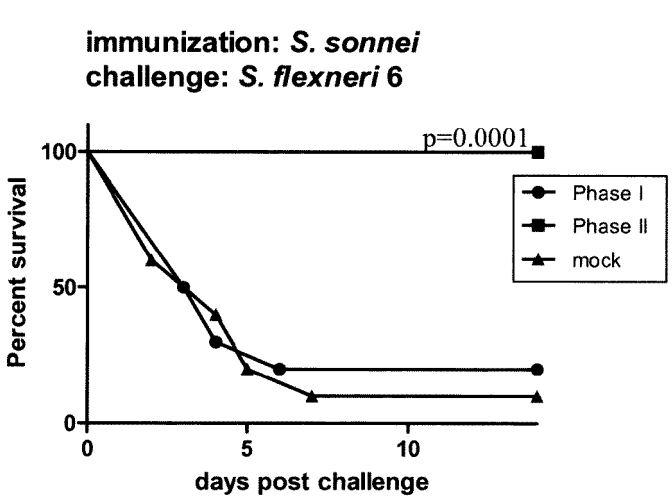

immunization: S. sonnei
challenge: S. flexneri 6
Percent survival
100
50
0
0
5
10
days post challenge
p=0.0001
Phase I
Phase II
mock

Fig. 2

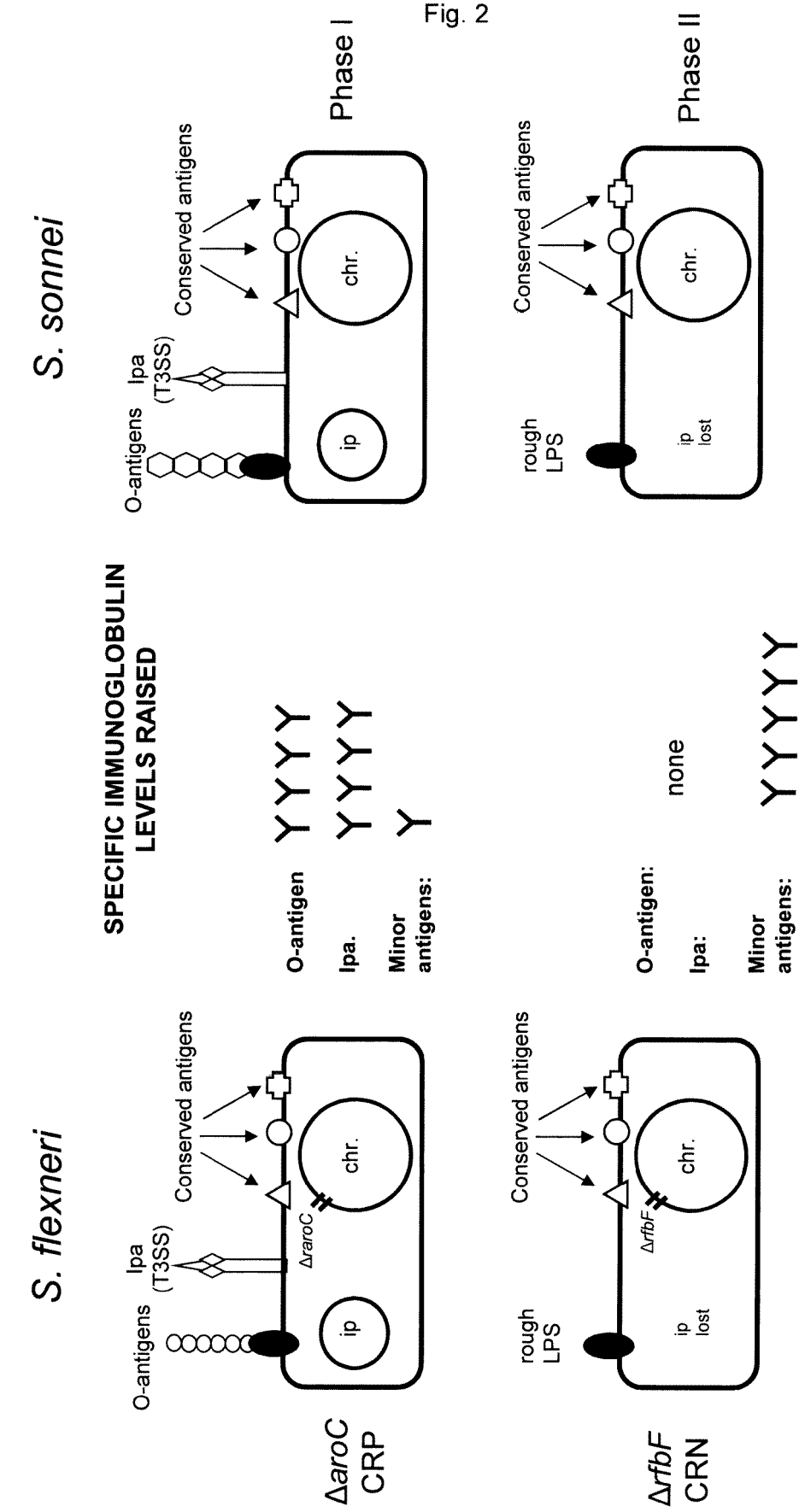

S. sonnei
Phase I
Phase II
O-antigens
Ipa (T3SS)
Conserved antigens
chr.
ip
rough LPS
ip lost
SPECIFIC IMMUNOGLOBULIN LEVELS RAISED
O-antigen
Ipa.
Minor antigens:
O-antigen:
Ipa:
Minor antigens:
none
S. flexneri
ΔaroC
CRP
ΔrfbF
CRN

Fig. 3

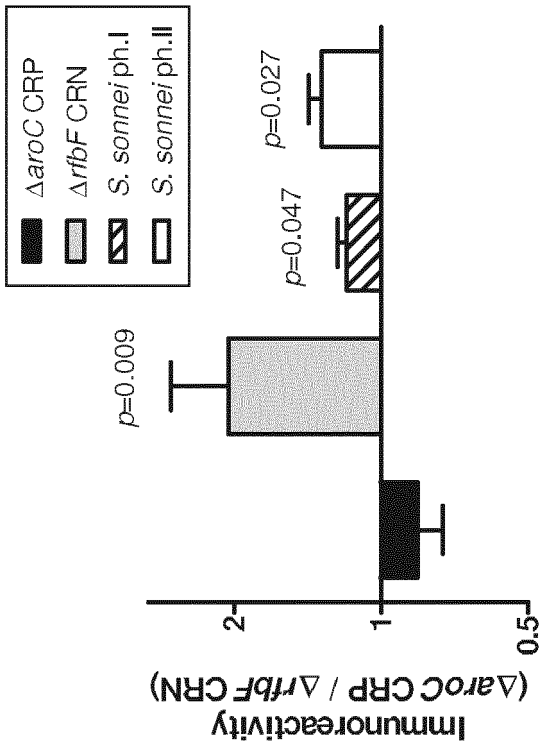
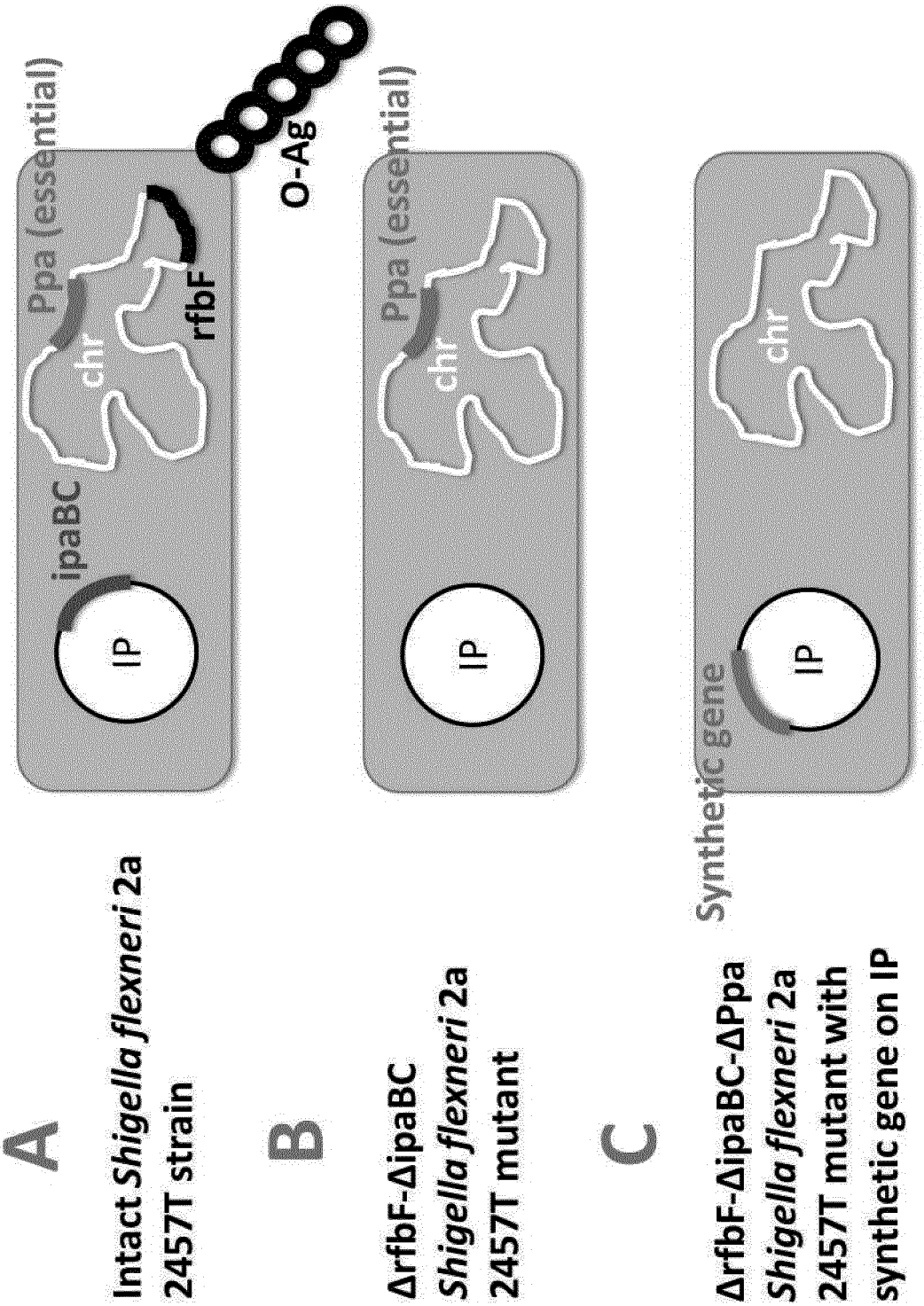

ΔaroC CRP
ΔrfbF CRN
S. sonnei ph.I
S. sonnei ph.II
p=0.009
p=0.047
p=0.027
2
1
0.5
Immunoreactivity
(ΔaroC CRP / ΔrfbF CRN)

Fig. 4

Oligo-nucleotide	Function	Oligonucleotid sequence [a]
aroCpKD-F	Amplification of PCR product used for the allelic exchange of *aroC*	5' CGC ACG GGC TGG CGC TCG GCT GCT GCA TCG TCG ATG GTG TTC C*GT GTA GGC TGG AGC TGC TTC* 3' (SEQ ID 3)
aroCpKD-R	Amplification of PCR product used for the allelic exchange of *aroC*	5' TAT CAG TCT TCA CAT CGG CAT TTT GCG CCC GCT GCC GTA A*CA TAT GAA TAT CCT CCT TAG TTC CTA TTA A* 3' (SEQ ID 4)
rfbFpKD-F	Amplification of PCR product used for the allelic exchange of *rfbF*	5' GAA TAG TAA TAT TTA CGC TGT CAT TGT GAC ATA TAA TCC CG*G TGT AGG CTG GAG CTG CTT C* 3' (SEQ ID 5)
rfbFpKD-R	Amplification of PCR product used for the allelic exchange of *rfbF*	5' GCA TTA TAA CGA CCG CCC CCA GTA ATT CCT CTT ATT CC*C ATA TGA ATA TCC TCC TTA GTT CCT AAT CC* 3' (SEQ ID 6)
aro-ko1	Confirmation of allelic exchange within *aroC*	5' GAG CCG TGA TGG CTG GAA ACA C 3' (SEQ ID 7)
aro-ko2	Confirmation of allelic exchange within *aroC*	5' AGC GCA ATC GCG GTT TTG TTC A 3' (SEQ ID 8)
rfbF-ko1	Confirmation of allelic exchange within *rfbF*	5' GGG TTA CTG GGT GCC GCA ATA TCC 3' (SEQ ID 9)
rfbF-ko2	Confirmation of allelic exchange within *rfbF*	5' CCT CAA TCC AGC ATT CGC CAT TAT ACG 3' (SEQ ID 10)

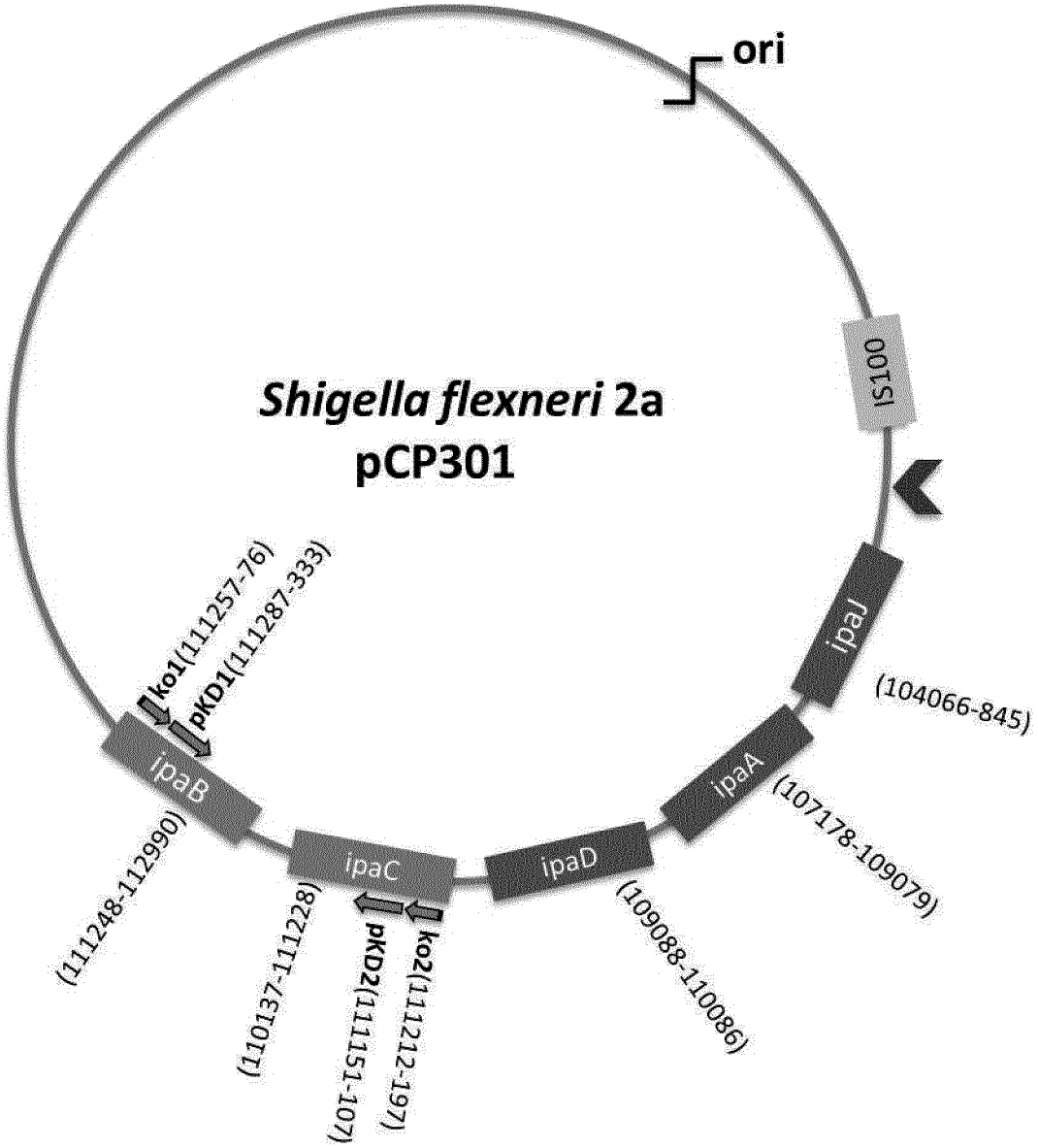
[a] The underlined sequence is specific to the helper plasmid used for the amplification of the antibiotic resistance cassette required for the mutagenesis

Fig. 5A, 5B, 5C


A
Intact Shigella flexneri 2a
2457T strain
Ppa (essential)
chr
ipaBC
IP
rfbF
O-Ag
B
ΔrfbF-ΔipaBC
Shigella flexneri 2a
2457T mutant
Ppa (essential)
chr
IP
C
ΔrfbF-ΔipaBC-ΔPpa
Shigella flexneri 2a
2457T mutant with
synthetic gene on IP
Synthetic gene
chr
IP

5

Fig. 6


ori
Shigella flexneri 2a
pCP301
IS100
ipaJ
(104066-845)
ipaA
(107178-109079)
ipaD
(109088-110086)
ipaC
(110137-111228)
pKD2(111151-107)
ko2(111212-197)
ipaB
(111248-112990)
ko1(111257-76)
pKD1(111287-333)

5

Fig. 7

>GP-P13F (SEQ ID 11)
GCTGCCGTGGTTCAAGTCGCGACTAATAAAAATAATCAGGTTGCCATGATTCAAT
GTACACCTTTCTCACATTCGTCTCCGGCATGAAAACGATGCACTCTTTCTTTATCG
CTTTCACTACACATTTTATCCTCGCATGGATGTTTTATAAAAAACATGATTGACATC
ATGTTGCATATAGGTTAAACAAAACAAGTGGCGTTATCTTTTTCCGGATTGTCTTCT
TGTATGATATATAAGTTTTCCTCGATGAATAAAGTAAAATGTTATGTTTTATTTACG
GCGTTACTATCCTCTCTATGTGCATACGGAGCTCCCCAGTCTATTACAGAACTATG
TTCGGAATATCGCAACACACAAATATATACGATAAATGACAAGATACTATCATATA
CGGAATCGATGGCAGGCAAAAGAGAAATGGTTATCATTACATTTAAGAGCGGCGC
AACATTTCAGGTCGAAGTCCCGGGCAGTCAACATATAGACTCCCAAAAAAAAGCC
ATTGAAAGGATGAAGGACACATTAAGAATCACATATCTGACCGAGACCAAAATTGA
TAAATTATGTGTATGGAATAATAAAACCCCCAATTCAATTGCGGCAATCAGTATGG
AAAACGGGCCGGGGCCCAATTCTTCTAACTACTGCTGTGAACTTTGTTGTAATTTT
GCCTGTACAGGATGTTACTAGTTTGCTTTAAAAGCATGTCTAATGCTAGGAACCTA
TATAACAACTACTGTACTTATACTAATGAGCCTTATGCTGCATTTGAAAAGGCGGT
AGAGGATGCAAT

>GP-P13F (SEQ ID 12)
MNKVKCYVLFTALLSSLCAYGAPQSITELCSEYRNTQIYTINDKILSYTESMAGKREMV
IITFKSGATFQVEVPGSQHIDSQKKAIERMKDTLRITYLTETKIDKLCVWNNKTPNSIAAI
SMENGPGPNSSNYCCELCCNFACTGCY

> GS-P13G (SEQ ID 13)
GCTGCCGTGGTTCAAGTCGCGACTAATAAAAATAATCAGGTTGCCATGATTCAAT
GTACACCTTTCTCACATTCGTCTCCGGCATGAAAACGATGCACTCTTTCTTTATCG
CTTTCACTACACATTTTATCCTCGCATGGATGTTTTATAAAAAACATGATTGACATC
ATGTTGCATATAGGTTAAACAAAACAAGTGGCGTTATCTTTTTCCGGATTGTCTTCT
TGTATGATATATAAGTTTTCCTCGATGAATAAAGTAAAATGTTATGTTTTATTTACG
GCGTTACTATCCTCTCTATGTGCATACGGAGCTCCCCAGTCTATTACAGAACTATG
TTCGGAATATCGCAACACACAAATATATACGATAAATGACAAGATACTATCATATA
CGGAATCGATGGCAGGCAAAAGAGAAATGGTTATCATTACATTTAAGAGCGGCGC
AACATTTCAGGTCGAAGTCCCGGGCAGTCAACATATAGACTCCCAAAAAAAAGCC
ATTGAAAGGATGAAGGACACATTAAGAATCACATATCTGACCGAGACCAAAATTGA
TAAATTATGTGTATGGAATAATAAAACCCCCAATTCAATTGCGGCAATCAGTATGG
AAAACGGCGGAGGTGGCTCCAATTCTTCTAACTACTGCTGTGAACTTTGTTGTAAT
GGCGCCTGTACAGGATGTTACTAGTTTGCTTTAAAAGCATGTCTAATGCTAGGAAC
CTATATAACAACTACTGTACTTATACTAATGAGCCTTATGCTGCATTTGAAAAGGC
GGTAGAGGATGCAAT

> GS-P13G (SEQ ID 14)
MNKVKCYVLFTALLSSLCAYGAPQSITELCSEYRNTQIYTINDKILSYTESMAGKREMV
IITFKSGATFQVEVPGSQHIDSQKKAIERMKDTLRITYLTETKIDKLCVWNNKTPNSIAAI
SMENGGGGSNSSNYCCELCCNGACTGCY

Fig. 7 (continued)

> GS-N12R (SEQ ID 15)

GCTGCCGTGGTTCAAGTCGCGACTAATAAAAATAATCAGGTTGCCATGATTCAAT
GTACACCTTTCTCACATTCGTCTCCGGCATGAAAACGATGCACTCTTTCTTTATCG
CTTTCACTACACATTTTATCCTCGCATGGATGTTTTATAAAAAACATGATTGACATC
ATGTTGCATATAGGTTAAACAAAACAAGTGGCGTTATCTTTTTCCGGATTGTCTTCT
TGTATGATATATAAGTTTTCCTCGATGAATAAAGTAAAATGTTATGTTTTATTTACG
GCGTTACTATCCTCTCTATGTGCATACGGAGCTCCCCAGTCTATTACAGAACTATG
TTCGGAATATCGCAACACACAAATATATACGATAAATGACAAGATACTATCATATA
CGGAATCGATGGCAGGCAAAAGAGAAATGGTTATCATTACATTTAAGAGCGGCGC
AACATTTCAGGTCGAAGTCCCGGGCAGTCAACATATAGACTCCCAAAAAAAAGCC
ATTGAAAGGATGAAGGACACATTAAGAATCACATATCTGACCGAGACCAAAATTGA
TAAATTATGTGTATGGAATAATAAAACCCCCAATTCAATTGCGGCAATCAGTATGG
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CCCTGCCTGTACAGGATGTTACTAGTTTGCTTTAAAAGCATGTCTAATGCTAGGAA
CCTATATAACAACTACTGTACTTATACTAATGAGCCTTATGCTGCATTTGAAAAGG
CGGTAGAGGATGCAAT

> GS-N12R (SEQ ID 16)

MNKVKCYVLFTALLSSLCAYGAPQSITELCSEYRNTQIYTINDKILSYTESMAGKREMV
IITFKSGATFQVEVPGSQHIDSQKKAIERMKDTLRITYLTETKIDKLCVWNNKTPNSIAAI
SMENGGGGSNSSNYCCELCCRPACTGCY

> GS-N12K (SEQ ID 17)

GCTGCCGTGGTTCAAGTCGCGACTAATAAAAATAATCAGGTTGCCATGATTCAAT
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CTTTCACTACACATTTTATCCTCGCATGGATGTTTTATAAAAAACATGATTGACATC
ATGTTGCATATAGGTTAAACAAAACAAGTGGCGTTATCTTTTTCCGGATTGTCTTCT
TGTATGATATATAAGTTTTCCTCGATGAATAAAGTAAAATGTTATGTTTTATTTACG
GCGTTACTATCCTCTCTATGTGCATACGGAGCTCCCCAGTCTATTACAGAACTATG
TTCGGAATATCGCAACACACAAATATATACGATAAATGACAAGATACTATCATATA
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CCTGCCTGTACAGGATGTTACTAGTTTGCTTTAAAAGCATGTCTAATGCTAGGAAC
CTATATAACAACTACTGTACTTATACTAATGAGCCTTATGCTGCATTTGAAAAGGC
GGTAGAGGATGCAAT

>-GS-N12K (SEQ ID 18)

MNKVKCYVLFTALLSSLCAYGAPQSITELCSEYRNTQIYTINDKILSYTESMAGKREMV
IITFKSGATFQVEVPGSQHIDSQKKAIERMKDTLRITYLTETKIDKLCVWNNKTPNSIAAI
SMENGGGGSNSSNYCCELCCKPACTGCY

Ipa co1 (SEQ ID 19)

GTAAGCACCACAACCACTGG

Fig. 7 (continued)

lpa co2 (SEQ ID 20)
CCAGCAATCTGACTGGCTGTCG

lpa pKD1 (SEQ ID 21)
GCCAAAATATTGGCTTCCACTGAGCTTGGAGACAATACTATCCAAGCCATATGAAT
ATCCTCCTTAGTTCCTAATCC

lpa pKD2 (SEQ ID 22)
GTATTAATTGATTTGTCGCTTGGGATGCTTCTTTAGATACTTGGGGTGTAGGCTGG
AGCTGCTTC

lpaBC (SEQ ID 23)
ATGCATAATGTAAGCACCACAACCACTGGTTTTCCTCTTGCCAAAATATTGGCTTC
CACTGAGCTTGGAGACAATACTATCCAAGCTGCAAATGATGCAGCTAACAAATTAT
TTTCTCTTACAATTGCTGATCTTACTGCTAACCAAAATATTAATACAACTAATGCAC
ACTCAACTTCAAATATATTAATCCCTGAACTTAAAGCACCAAAGTCATTAAATGCAA
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GCAGCTGTTGGTTTAGCTCTTATGGTTACGGATGCTATAGTACAAGCAGCGACCG
GCAATTCCTTCATGGAACAAGCCCTGAATCCGATCATGAAAGCAGTCATTGAACC
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ACTCGAAAAAAGCCAAAATGATTGGCTCTATTCTGGGGGCAATCGCAGGCGCTCT
TGTCCTAGTTGCAGCAGTCGTTCTCGTAGCCACTGTTGGTAAACAGGCAGCAGCA
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CTAGCAGACCTGACATTATCGAAATATCAAGTTGAACAACTGTCAAAATATATCAG
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CCCAGATTTTATATACAGATATATCCACAAAACAAACTCAAAGTTCTTCCGAAACAC
AAAAATCACAAAATTATCAGCAGATTGCAGCGCATATTCCACTTAATGTCGGTAAA
AATCCCGTATTAACAACCACATTAAATGATGATCAACTTTTAAAGTTATCAGAGCAG
GTTCAGCATGATTCAGAAATCATTGCTCGCCTTACTGACAAAAAGATGAAAGATCG

Fig. 7 (continued)

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CTGCAGAAACTAAATTGGGCTCTCAATTGTCATTGATTGCGTTCGATGCTACAAAA
TCAGCTGCAGAGAACATTGTTCGGCAAGGCCTGGCAGCCCTATCATCAAGCATTA
CTGGAGCAGTCACACAAGTAGGTATAACGGGTATCGGTGCCAAAAAAACGCATTC
AGGGATTAGCGACCAAAAAGGAGCCTTAAGAAAGAACCTTGCCACTGCTCAATCT
CTTGAAAAAGAGCTTGCAGGTTCTAAATTAGGGTTAAATAAACAAATAGATACAAA
TATCACCTCACCACAAACTAACTCTAGCACAAAATTTTTAGGTAAAAATAAACTGG
CGCCAGATAATATATCCCTGTCAACTGAACATAAAACTTCTCTTAGTTCTCCCGAT
ATTTCTTTGCAGGATAAAATTGACACCCAGAGAAGAACTTACGAGCTCAATACCCT
TTCTGCGCAGCAAAAACAAAACATTGGCCGTGCAACAATGGAAACATCAGCCGTT
GCTGGTAATATATCCACATCAGGAGGGCGTTATGCATCTGCTCTTGAAGAAGAAG
AACAACTAATCAGTCAGGCCAGCAGTAAACAAGCAGAGGAAGCATCCCAAGTATC
TAAAGAAGCATCCCAAGCGACAAATCAATTAATACAAAAATTATTGAATATAATTGA
CAGCATCAACCAATCAAAGAATTCGACAGCCAGTCAGATTGCTGGTAACATTCGA
GCTTAA

PpA (SEQ ID: 24)

AAGTAAATAAAACGTTAATCACAAGTTTGTAATCGCTTTCATCTCACTATGAAAAAT
GCGGCTACGGTTATGGATTTTCCTGCTCTGTATACCGTCTTAAAACTGGCGAAAAA
GGAAAATGAAGACGAAAACAAGCAAAGACATTCGGCGCGAGTTGGCTATAATACT
TGGCACTTGTTTGCCACATATTTTTAAAGGAAACAGACATGAGCTTACTCAACGTC
CCTGCGGGTAAAGATCTGCCGGAAGACATCTACGTTGTTATTGAGATCCCGGCTA
ACGCAGATCCGATCAAATACGAAATCGACAAAGAGAGCGGCGCACTGTTCGTTGA
CCGCTTCATGTCCACCGCGATGTTCTATCCGTGCAACTACGGTTACATCAACCAC
ACCCTGTCTCTGGACGGTGACCCGGTTGACGTACTGGTCCCGACTCCGTACCCG
CTGCAGCCTGGTTCTGTGATCCGTTGCCGTCCGGTTGCCGTTCTGAAAATGACCG
ACGAAGCCGGTGAAGATGCAAAACTGGTTGCGGTTCCGCACAGCAAGCTGAGCA
AAGAATACGATCACATTAAAGACGTAAACGATCTGCCTGAACTGCTGAAAGCGCA
AATCGCTCACTTCTTCGAGCACTACAAAGACCTCGAAAAAGGCAAGTGGGTGAAA
GTTGAAGGTTGGGAAAACGCAGAAGCCGCTAAAGCTGAAATCGTTGCTTCCTTCG
AGCGCGCAAAGAATAAATAAGTTCTTCTAGCGCAATAACCCTGAACGCCGGGCTT
CGGTTAGTAAGGGTTTTTTTATGCCCGCGATAAATAAACTCTCTATTCCACCATCA
TTATTCTCAGCGGTTGCAAGGCTTGAACGGTAAGAACAAGCAAACCCGACCACCA
TTTTGCTGTTCATAGCCACTTGCTGGAAGTTAGCCGACCTCACTCATACTCACCG

ppa pKD-F (SEQ ID: 25)
TTATTATTGGTGAAAAGATGTTCGCGAAAAAACTATAGACAATTCGTTATGTAACG
GATTGCGTTACATCGTGTAGGCTGGAGCTGCTTC

ppa pKD-R (SEQ ID: 26)
TCCATATCTGCAATCGCATAAAAAACTCTGCTGGCGTTCACAAATGTGCAGGGGT
AAAACGGGGGCACGCCATATGAATATCCTCCTTAGTTCCTAATCC

ppa ko1 (SEQ ID: 27)
CATAGGGTTGTCCTCGTCGGGG

Fig. 7 (continued)

5

ppa ko2 (SEQ ID: 28)
GTT TTA TGC GAT GTA TCT CGC G

Linker (SEQ ID 29)
GGGGS

Fig. 8

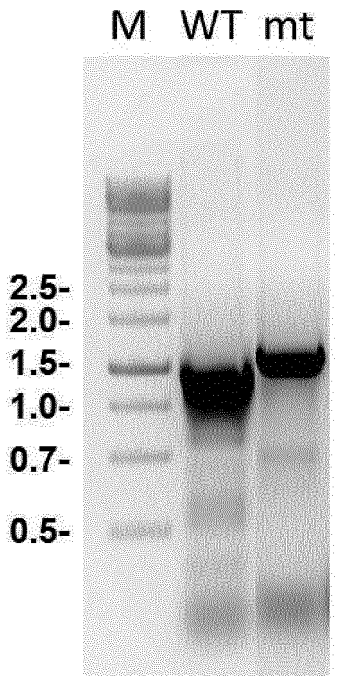


Fig. 9

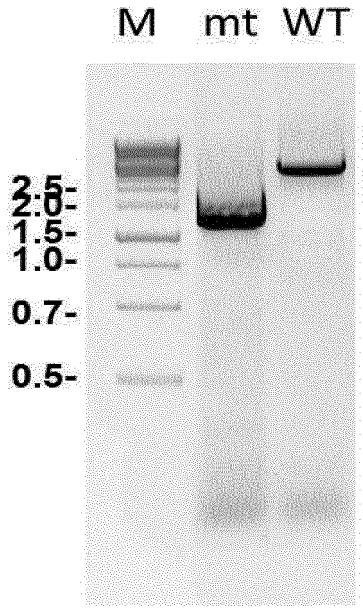


Fig. 10A

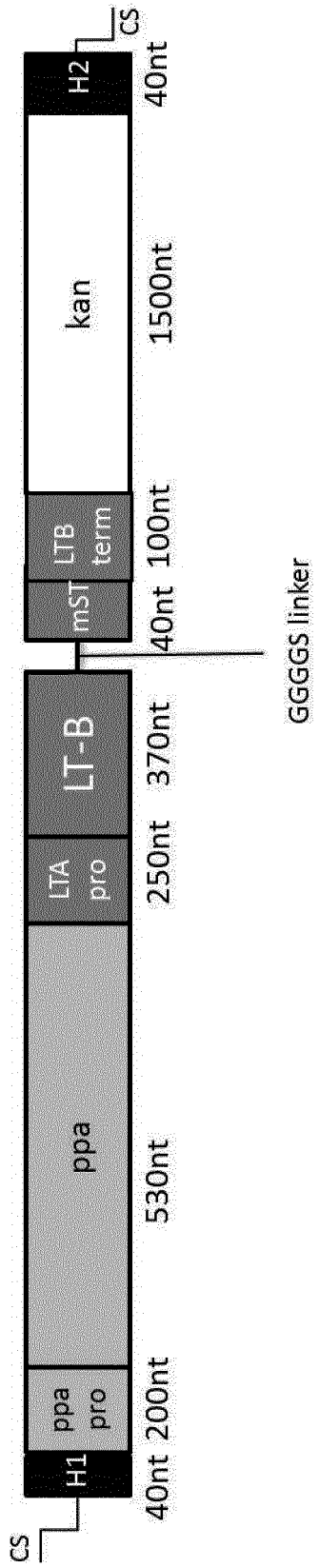

CS
H1
ppa pro
ppa
LTA pro
LT-B
GGGGS linker
nST
LTB term
kan
H2
CS
40nt
200nt
530nt
250nt
370nt
40nt
100nt
1500nt
40nt

Fig. 10B

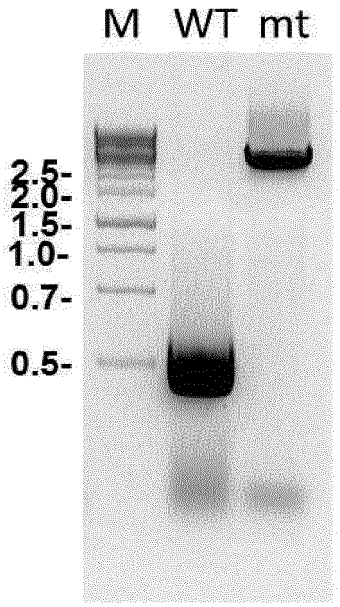


Fig. 10C

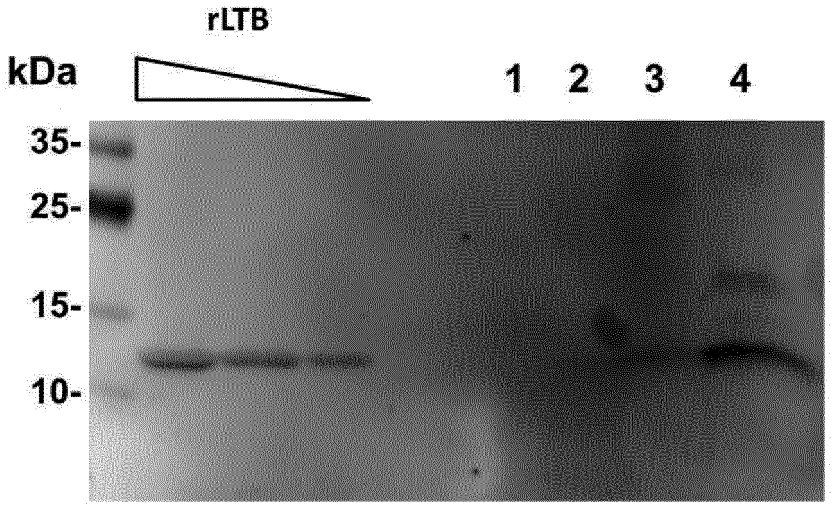
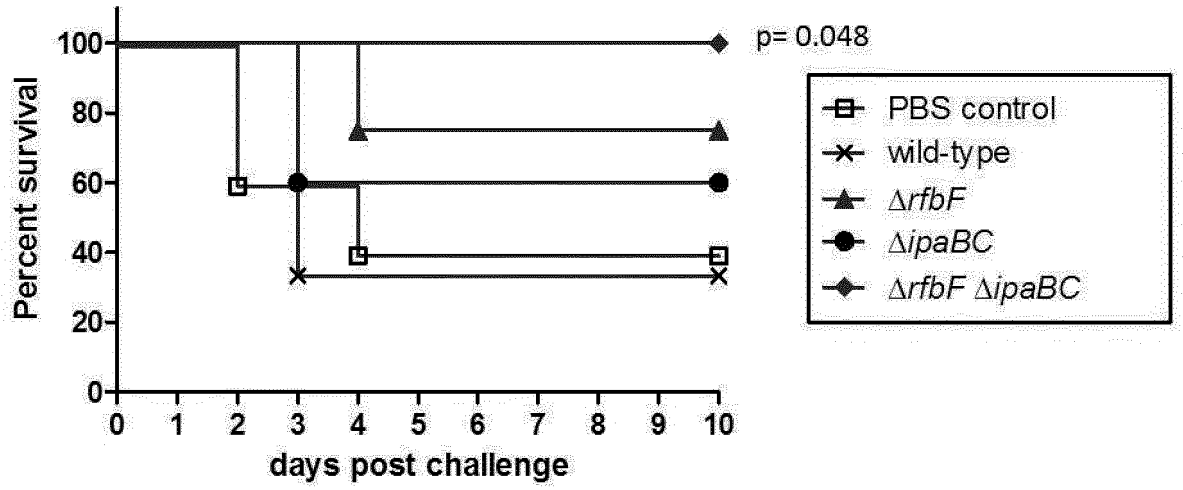


Fig. 11


p= 0.048
PBS control
wild-type
ΔrfbF
ΔipaBC
ΔrfbF ΔipaBC
Percent survival
100
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10
days post challenge

5