

Table S1: The primers used for PCR.

Gene	Primer (5'-3')
bp26-N-F	ATATGAGCTCGTGTGTGCGCCTGAAGCGCAAT
bp26-N-R	CACCACACGGCCAAGGACGAGCATGATTGTGGAAAATGA
bp26-C-F	ACAATCATGCTCGTCCTTGGCCGTGTGGTGGAAATC
bp26-C-R	CGGCTGCAGTTTCCGTTTCATCATTTGG
bp26-J-F	ATGAACACTCGTGCTAGCAATTTTCTCG
bp26-J-R	TTACTTGATTTCAAAAACGACATTGACC
wboA-N-F	TATTGAGCTCTTGCAGACGGAGCAGCCCTCCAC
wboA-N-R	CGCATACTCCTTGTCACTGATTTCCCGTGTCTACTC
wboA-C-F	ACACGGGAAATCAGTGACAAGGAGTATGCGGAGCTT
wboA-C-R	CGGCTGCAGGAACTTATCTCTCAACTTCCAT
wboA-J-F	TGTGGCCAATGTCACCACAAATG
wboA-J-R	AGTCGTTTCAAAGCGTTGGACATC

Table S2: Immunization details of various experiments in sheep.

Experiment	Species	Doses	Times
Regular dose, single immunization	sheep	1×10 ⁹ CFU/1ml	1 time
Regular dose, multiple immunization	sheep	1×10 ⁹ CFU/1ml	3 times with a 7-days interval
High dose, single immunization	sheep	5×10 ⁹ CFU/1ml	1 time
Over dose, single immunization	Pregnant sheep	1×10 ¹⁰ CFU/1ml	1 time

Table S3: Key resources table

Reagent or resource	Source	Identifier
Bacterial strains		
RM6Δbp26ΔwboA (RM6)	huaxishengwu	N/A
Brucella Suis strain S2	AOLONG	Cat#CVCC70502
Chemicals, peptides, and recombinant proteins		
0.9% sodium chloride injection		N/A
Rose bengal agglutination antigen	IVDC	20180701
Rough Brucella agglutination antigen	IVDC	20190102
Smooth Brucella positive serum (S)	IVDC	N/A
Rough Brucella positive serum (R)	IVDC	N/A
A、M single serum lyophilized product	IVDC	N/A
Broth medium	Rongcheng	20170609
sodium carboxymethylcellulose	Sinopharm	20140122-500
Glucose	Sinopharm	2017050221
Sucrose	Sinopharm	20161010
Gelatin	Sinopharm	20141126
Thiourea	Sinopharm	20150304
Tryptone soy agar (TSA) medium	BD	Cat#211043
Tryptone soy broth (TSB) medium	BD	Cat#257107

Ex Taq DNA polymerase	TaKaRa	N/A
DNA Marker	TaKaRa	N/A
Brucella selective supplement	Hopebio	20170510
Powdered agar	Sinopharm	20160406
Ex Taq Polymerase kit	TaKaRa	Lot KA6501AA
Bacterial DNA special extraction kit	Sinopharm	N/A
agarose gel dna extraction kit	Sinopharm	N/A
Plasmid extraction kit	Sinopharm	N/A
RNAprep pure tissue kit	Tiagen	Cat#DP431
Gram staining	bjbiotopped	N/A
lead acetate paper strips	Science, Shanghai	N/A
Thionin	Sangon bio	N/A
Basic fuchsin	Sangon bio	N/A
Acridiflavine	Sangon bio	N/A
Crystal violet staining	Sangon bio	N/A
Experimental models: Organisms/strains		
Sheep	This paper	N/A
Pregnant sheep	This paper	N/A
Mouse: Bal b/C	Charles river	N/A
Oligonucleotides		
Primers	Table S1	N/A
Software and algorithms		
GraphPad 9.0	OriginLab	https://www.graphpad.com/scientific-software/prism

Table S4: Depleted Sequences.

bp26	
1	GCGCCTTTCA GCCTGCCCGC TTTTCGCACAG GAGAATCAGA TGACGACGCA GCCCGCGCGC
61	ATCGCCGTCA CCGGGGAAGG CATGATGACG GCCTCGCCCG ATATGGCCAT TCTCAATCTC
121	TCGGTGCTAC GCCAGGCAAA GACCGCGCGC GAAGCCATGA CCGCGAATAA TGAAGCCATG
181	ACAAAAGTGC TCGATGCCAT GAAGAAGGCC GGCATCGAAG ATCGCGATCT CCAGACAGGC
241	GGCATCAATA TCCAGCCGAT TTATGTCTAT CCTGACGACA AGAACAACCT GAAAGAGCCT
301	ACCATCACCG GCTATTTCTGT ATCCACCAGT CTCACGGTTC GCGTGCGCGA ACTGGCCAAT
361	GTTGGAAAAA TTTTGGATGA ATCCGTCACG CTCGGTGTTA ATCAGGGCGG TGATTTGAAC
421	CTGGTCAATG ATAATCCCTC CGCCGTGATC AACGAGGCGC GCAAGCGCGC AGTGGCCAAT
481	GCCATTGCCA AGGCGAAGAC GCTTGCCGAC GCTGCAGGCG TGGGG
wboA	
1	ATGGCTCCGA GACATATTAC AGTTATCCTA CCAGCTAAGT ACCGAGGCGG AAGTCTTCGA
61	GTTACGAAGA ATATCGTTTCG AATGCTTTTG AAGGGAAGTC AGAATTATGG TGAACAGTGT
121	CAAGTTAGAT TGGCAGTACG TGCCGATACC TACGATATTG GGGAGGAGTT TCGTGATCTT
181	ATCGATAATG GTGTAGAGGT TCGGGAAATA TCATTCAAAG AAGTTCCTCC AGAAGATGTT
241	AACAATGCTA ACTATTTCCA AGGTAGAAAT ATCGACCTAC AGTCGAGAAC CTATTGGCTA
301	ATGGAGGATG GCCAAAACAA CTGTGCCGAT AGTGACCTTT GGCTAGTTGT ATCCTACTCT

361 GTAGAGTATC CTATTGCCCC GATAAGGCCG AACTGATAT TTGCCACCGA TTTCATTCAA
421 AGGTACGTAC CTGATATTAT TTGGCCACCA CGGCCCGGTG AGGGGGATGC TGAGGCTCTT
481 GCGTTCCTTAC GACAATCAGA CGGCGTACTA GCTACAACAC CACACACGCG GCTGGATGCG
541 ATTCATACG CTGGCTTACC TGCCTCCAAA GTTTATCTTG CTCCGATGGA GTTTGACCCG
601 ACGTTTTTGG ATCGTTACCG GTCAGTGTCT AAGGTTAAGG AACCTATTT CCTTTGGCCA
661 ACCAACCCAA ATGCTCACAA AAACCATGCA AAAGCGTTC AAGCGCTAGA CCTATATTAC
721 GGCAAATAA AGGTAAGAT AAAGACAAAG ATAGTCGGTG TGAGTAGTGT GCGGATGGAC
781 CCATCCATC GATGGCAGGC CAAGTACGAA AATAAGGCTT ATGTGAAATC TGTACGGGAA
841 ATTGTTGCGG GTCTCGACAA CCTGAAAAGC AATGTTGAGT TCGCTGGTGA GGTTCGG
