

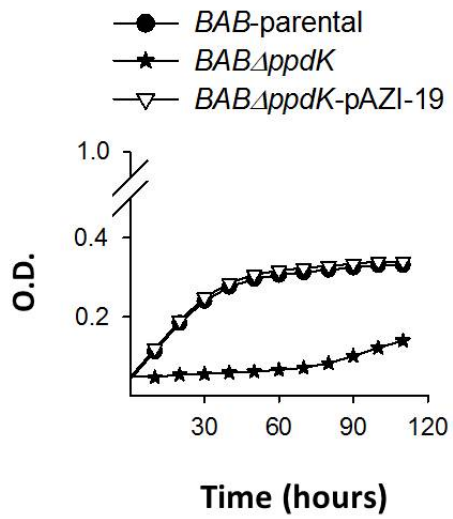
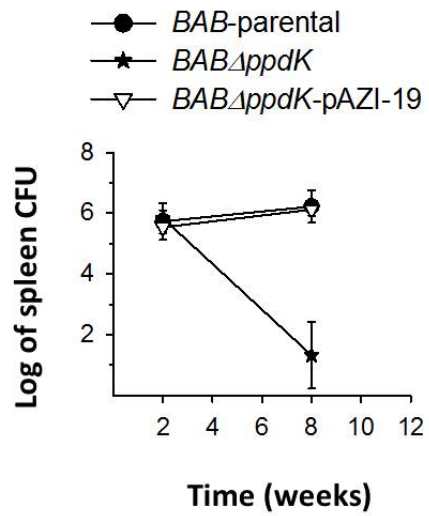
**A****B**

FIG. S1. (A) Growth curves in Glycerol-Lactate-Glutamate of *BAB*-parental, *BAB*Δ*ppdK* and *BAB*Δ*ppdK*-pAZI-19 (containing the complete *ppdK* gene). Each point represents the mean of triplicate samples (error bars are within the size of the symbols). The experiment was repeated three times with similar results. (B) Bacterial multiplication of *BAB*-parental, *BAB*Δ*ppdK* and *BAB*Δ*ppdK*-pAZI-19 in BALB/c mice. Each point is the mean  $\pm$  standard deviation ( $n=5$ ) of the Log of CFU.

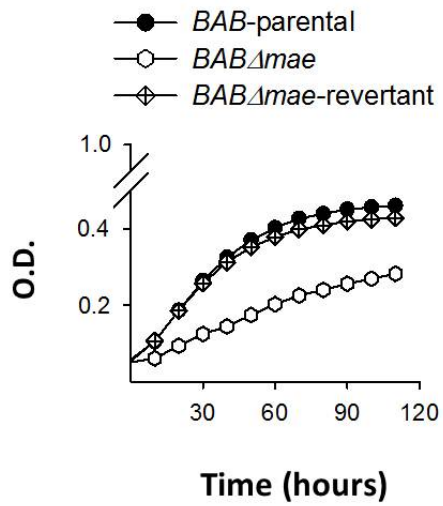
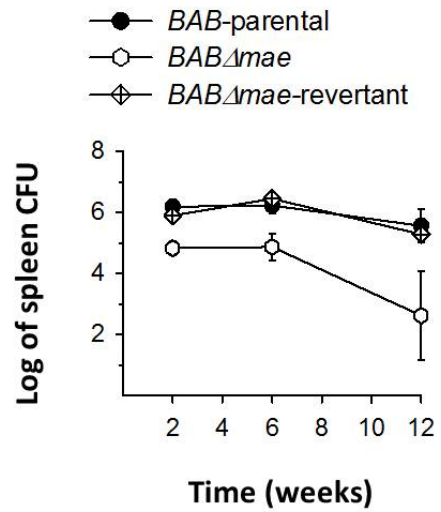
**A****B**

FIG. S2. (A) Growth curves in Glycerol-Lactate-Glutamate of *BAB*-parental, *BAB* $\Delta$ *mae* and *BAB* $\Delta$ *mae*-revertant. Each point represents the mean of triplicate samples (error bars are within the size of the symbols). The experiment was repeated three times with similar results. (B) Bacterial multiplication of *BAB*-parental, *BAB* $\Delta$ *mae* and *BAB* $\Delta$ *mae*-revertant in BALB/c mice. Each point is the mean  $\pm$  standard deviation ( $n=5$ ) of the Log of CFU.

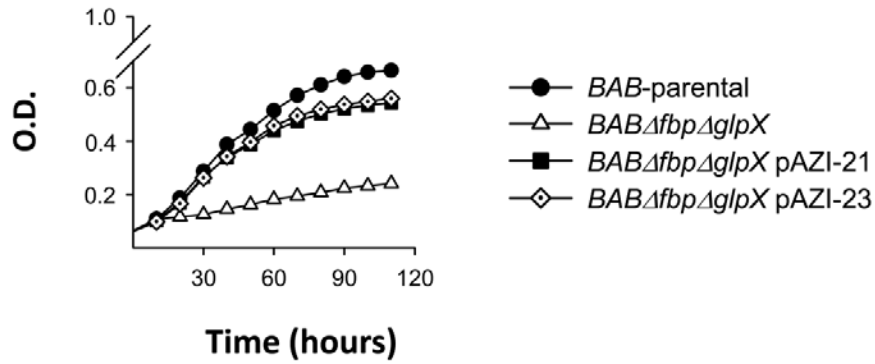


FIG. S3. Growth curves in Glycerol-Lactate-Glutamate of *BAB*-parental, *BABΔfbpΔglpX*, *BABΔfbpΔglpX* pAZI-21 (containing the complete *fbp* gene) and *BABΔfbpΔglpX* pAZI-23 (containing the complete *glpX* gene). Each point represents the mean of triplicate samples (error bars are within the size of the symbols). The experiment was repeated three times with similar results.

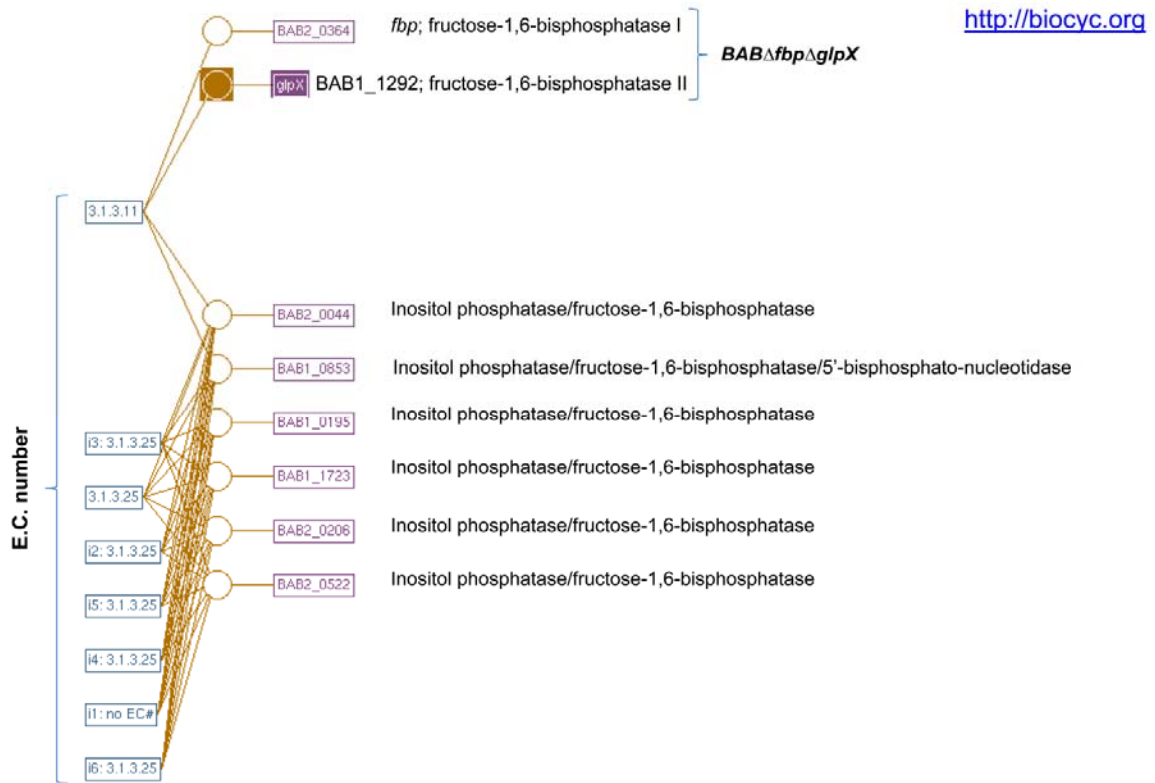


FIG. S4. Putative phosphatases close to Fbp and GlpX in the *B. abortus* 2308 genome.

**Table S1. Bacterial strains and plasmids.**

Strain or plasmid	Characteristics	Reference /Source
<b><i>Brucella abortus</i></b>		
<i>B. abortus</i> 2308	Isolated in 1942. Used in experimental infection for over 30 years (passaged twice each in cows and guinea pigs, but only four times in total <i>in vitro</i> )	(1)
<i>B. abortus</i> 2308 Nal <sup>R</sup> (BAB-parental)	Wild type, virulent, biotype 1, smooth LPS, Nal <sup>R</sup> spontaneous mutant of strain 2308	(2)
BABΔ <i>fbp</i>	2308 Nal <sup>R</sup> <i>fbp</i> <sub>Δ15-326</sub>	This work
BABΔ <i>glpX</i>	2308 Nal <sup>R</sup> <i>glpX</i> <sub>Δ21-205</sub>	This work
BABΔ <i>fbp</i> Δ <i>glpX</i>	2308 Nal <sup>R</sup> <i>fbp</i> <sub>Δ15-326</sub> <i>glpX</i> <sub>Δ21-205</sub>	This work
BABΔ <i>pckA</i>	2308 Nal <sup>R</sup> <i>pckA</i> <sub>Δ14-454</sub>	This work
BABΔ <i>ppdK</i>	2308 Nal <sup>R</sup> <i>ppdK</i> <sub>Δ35-859</sub>	This work
BABΔ <i>ppdK-II</i>	2308 Nal <sup>R</sup> <i>ppdK</i> <sub>Δ35-887</sub>	This work
BABΔ <i>pckA</i> Δ <i>ppdK</i>	2308 Nal <sup>R</sup> <i>pckA</i> <sub>Δ14-454</sub> <i>ppdK</i> <sub>Δ35-887</sub>	This work
BABΔ <i>mae</i>	2308 Nal <sup>R</sup> <i>mae</i> <sub>Δ26-700</sub>	This work
BABΔ <i>aceA</i>	2308 Nal <sup>R</sup> <i>aceA</i> <sub>Δ11-419</sub>	This work
<i>virB</i>	2308 Nal <sup>R</sup> mutant deleted of <i>virB10</i>	(3)
<b><i>Escherichia coli</i></b>		
S17-λ <i>pir</i>	Mating strain with plasmid RP4 inserted into the chromosome (Tpr Smr <i>recA thi hsdR</i> M+, lambda <i>pir</i> phage lysogen RP4::2-Tc::Mu:Km Tn7)	(4,5)
TOP10F'	F' <i>lacIq Tn 10</i> (Tetr) <i>mcrA</i> Δ( <i>mrr-hsdRMS-mcrBC</i> ) 80 <i>lacZ</i> ΔM15 Δ <i>lacX74 recA1alaD139</i> Δ ( <i>ara-leu</i> )7697 <i>galU galK rpsL endA1 nupG</i>	Invitrogen
One shot OMNIMAX™	F' [ <i>proAB+ lacIq lacZ</i> ΔM15 <i>Tn10</i> (Tetr) Δ( <i>ccdAB</i> )] <i>mcrA</i> Δ( <i>mrr-hsdRMS-mcrBC</i> ) φ80( <i>lacZ</i> )ΔM15 Δ( <i>lacZYA-argF</i> ) U169 <i>endA1 recA1 supE44 thi-1 gyrA96 relA1 tonA panD</i>	Invitrogen
<b>Plasmid</b>		
pCR 2.1	Cloning vector, Km <sup>R</sup>	Invitrogen
pJQKm	Suicide vector, Km <sup>R</sup> , Sac <sup>S</sup>	(6)
pBBR1MCS1	Broad-host-range cloning vector, high copy number, Cm <sup>R</sup>	(7)
pRH001	Derivative of pMR10, Km <sup>R</sup> ; Cm <sup>R</sup>	(8)
pGEM®-T Easy	Cloning vector, Amp <sup>R</sup>	Promega
pSKOriTKm <i>luxAB</i>	Derivative of de pSKOriTKm <sup>R</sup> containing <i>luxAB</i> without promoter	(9)
pAZI-1	396 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABfbp</i> deletion allele, generated by PCR and cloned into pCR2.1	This work
pAZI-2	<i>Bam</i> HI/ <i>Xba</i> I fragment from pAZI-1 cloned into the corresponding sites of pJQKm	This work
pAZI-3	1150 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABglpX</i> deletion allele, generated by PCR and cloned into pCR2.1	This work
pAZI-4	<i>Bam</i> HI/ <i>Xba</i> I fragment from pAZI-3 cloned into the corresponding sites of pJQKm	This work
pAZI-5	538 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABpckA</i> deletion allele, generated by PCR and cloned into pCR2.1	This work
pAZI-6	<i>Bam</i> HI/ <i>Xba</i> I fragment from pAZI-5 cloned into the corresponding sites of pJQKm	This work
pAZI-7	516 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABaceA</i> deletion allele, generated by PCR and cloned into pCR2.1	This work
pAZI-8	<i>Bam</i> HI/ <i>Xba</i> I fragment from pAZI-7 cloned into the corresponding sites of pJQKm	This work
pMZI-1	508 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABppdK</i> deletion allele, generated by PCR and cloned into pCR2.1	This work
pMZI-2	<i>Bam</i> HI/ <i>Xba</i> I fragment from pMZI-1 cloned into the corresponding sites of pJQKm	This work
pMZI-3	717 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABmae</i> deletion allele, generated by PCR and cloned into pCR2.1	This work
pMZI-4	<i>Bam</i> HI/ <i>Xba</i> I fragment from pMZI-3 cloned into the corresponding sites of pJQKm	This work
pAZI-9	509 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABppdK</i> deletion allele, generated by PCR and cloned into pCR2.1	This work

pAZI-10	<i>Bam</i> HI/ <i>Xba</i> I fragment from pAZI-9 cloned into the corresponding sites of pJQKm	This work
pAZI-17	382 bp of <i>B. abortus</i> chromosomal DNA containing <i>BABaceA</i> promoter and ATG generated by PCR and cloned into <i>pGEM®-T Easy</i>	This work
pAZI-18 pDONR201- BME11436	<i>Bam</i> HI/ <i>Xba</i> I fragment from pAZI-17 cloned into pSKoriTKmLuxAB <i>B. melitensis</i> 16M chromosomal DNA containing the complete ORF BME11436 ( <i>ppdK</i> ), generated by PCR and cloned into pDONR201 (Invitrogen)	This work (10)
pAZI-19	attL1-attL2 fragment of pDONR201-BME11436 (containing the complete <i>ppdK</i> gene) cloned into the attR1-attR2 sites of pRH001	This work
pAZI-20	1388 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABfbp</i> gene, generated by PCR and cloned into pCR2.1	This work
pAZI-21	<i>Bam</i> HI/ <i>Xho</i> I fragment from pAZI-20 cloned into the corresponding sites of pBBR1MCS1	This work
pAZI-22	1705 bp of <i>B. abortus</i> chromosomal DNA containing the <i>BABglpX</i> gene, generated by PCR and cloned into pCR2.1	This work
pAZI-23	<i>Bam</i> HI/ <i>Xho</i> I fragment from pAZI-22 cloned into the corresponding sites of pBBR1MCS1	This work

## REFERENCES

1. **Verstrete, D. R., M. T. Creasy, N. T. Caveney, C. L. Baldwin, M. W. Blab, and A. J. Winter.** 1982. Outer membrane proteins of *Brucella abortus*: isolation and characterization. *Infect.Immun.* **35**:979-989.
2. **Sangari, F. and J. Aguero.** 1991. Mutagenesis of *Brucella abortus*: comparative efficiency of three transposon delivery systems. *Microb.Pathog.* **11**:443-446.
3. **Sieira, R., D. J. Comerçi, D. O. Sanchez, and R. A. Ugalde.** 2000. A homologue of an operon required for DNA transfer in *Agrobacterium* is required in *Brucella abortus* for virulence and intracellular multiplication. *J.Bacteriol.* **182**:4849-4855.
4. **Simon, L. D., B. Randolph, N. Irwin, and G. Binkowski.** 1983. Stabilization of proteins by a bacteriophage T4 gene cloned in *Escherichia coli*. *Proc.Natl.Acad.Sci.U.S.A* **80**:2059-2062.
5. **Miller, V. L. and J. J. Mekalanos.** 1988. A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*. *J.Bacteriol.* **170**:2575-2583.
6. **Scupham, A. J. and E. W. Triplett.** 1997. Isolation and characterization of the UDP-glucose 4'-epimerase-encoding gene, *galE*, from *Brucella abortus* 2308. *Gene* **202**:53-59.
7. **Kovach, M. E., Phillips, R. W., Elzer, P. H., Roop, R. M., and Peterson, K. M.** 1994. pBBR1MCS1: a broad-host-range cloning vector. *Biotechniques.* **16**:800-802.
8. **Hallez, R., J. J. Letesson, J. Vandehaute, and X. De Bolle.** 2007. Gateway-based destination vectors for functional analyses of bacterial ORFeomes: application to the Min system in *Brucella abortus*. *Appl.Enviro.Microbiol.* **73**:1375-1379.
9. **Conde-Álvarez, R.** A study on the role of phosphatidylcholine and inner lipopolysaccharide sections in *Brucella* virulence. 2008.
10. **Dricot, A., J. F. Rual, P. Lamesch, N. Bertin, D. Dupuy, T. Hao, C. Lambert, R. Hallez, J. M. Delroisse, J. Vandehaute, I. López-Goñi, I. Moriyón, J. M. García-Lobo, F. J. Sangari, A. P. MacMillan, S. J. Cutler, A. M. Whatmore, S. Bozak, R. Sequerra, L. Doucette-Stamm, M. Vidal, D. E. Hill, J. J. Letesson, and X. De Bolle.** 2004. Generation of the *Brucella melitensis* ORFeome version 1.1. *Genome Res* **14**:2201-2206.