

Supplementary Information

For Servais *et al*, "Lipopolysaccharide synthesis and traffic in the envelope of the pathogen *Brucella abortus*"

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Figure S8 | Wzm appears dispersed surrounding the bacterial cell

Table S1 | List of strains used in this study.

| Name | Genotype | Reference |
|------------------------------------------------------|------------------------------------------------------------------------------------------------|------------------------------------------------------------|
| <i>Brucella abortus</i> 544 strains | | |
| Wild type (WT) | <i>B. abortus</i> 544, NaI ^R | J.-M.Verger, INRA, Tours |
| <i>rgsE-mNG pdhS-mCherry</i> | <i>B. abortus</i> 544 <i>rgsE-mNG pdhS::pSK kan pdhs-mCherry</i> | This study |
| <i>lptD-AGDP</i> | <i>B. abortus</i> 544 <i>lptD-AGDP-G4-3Flag</i> | V. Vassen, PhD thesis, UNamur, BE |
| <i>mNG-lptC pdhS-mCherry</i> | <i>B. abortus</i> 544 <i>mNG-lptC pdhS::pSK kan pdhs-mCherry</i> | This study |
| <i>mNG-lptF pdhS-mCherry</i> | <i>B. abortus</i> 544 <i>mNG-lptF pdhS::pSK kan pdhs-mCherry</i> | This study |
| <i>msbA-mNG pdhS-mCherry</i> | <i>B. abortus</i> 544 <i>msbA-mNG pdhS::pSK kan pdhs-mCherry</i> | This study |
| <i>pSK_msbAmNG pKS popZ-mCherry</i> | <i>B. abortus</i> 544 <i>msbA::pSK kan msbA-mNG popZ::pSK kan popZ-mCherry</i> | This study |
| <i>pSK_msbA_{E491A}-mNG pKS popZ-mCherry</i> | <i>B. abortus</i> 544 <i>msbA::pSK kan msbA_{E491A}-mNG popZ::pSK kan popZ-mCherry</i> | This study |
| <i>Δgmd</i> | <i>B. abortus</i> 544 <i>Δgmd</i> | ¹ |
| <i>ΔwaaL</i> | <i>B. abortus</i> 544 <i>ΔwaaL</i> | This study |
| <i>ΔgmdΔwada</i> | <i>B. abortus</i> 544 <i>ΔgmdΔwada</i> | This study |
| <i>ΔgmdΔOAg-lig</i> | <i>B. abortus</i> 544 <i>ΔgmdΔOAg-lig</i> | This study |
| <i>Δgmd pBBRI gmd</i> | <i>B. abortus</i> 544 <i>Δgmd pBBRI_gmd</i> | This study |
| <i>ΔgmdΔwada pBBRI gmd</i> | <i>B. abortus</i> 544 <i>ΔgmdΔwada pBBRI_gmd</i> | This study |
| <i>ΔgmdΔOAg-lig pBBRI gmd</i> | <i>B. abortus</i> 544 <i>ΔgmdΔOAg-lig pBBRI_gmd</i> | This study |
| <i>mNG-wada</i> | <i>B. abortus</i> 544 <i>mNG-wada</i> | This study |
| <i>wada-mNG</i> | <i>B. abortus</i> 544 <i>wada-mNG</i> | This study |
| <i>mNG-wzm</i> | <i>B. abortus</i> 544 <i>mNG-wzm</i> | This study |
| <i>Brucella melitensis</i> 16M | | |
| Wild type (WT) | <i>B. melitensis</i> 16M, NaI ^R | A. Macmillan, Central Veterinary Laboratory, Weybridge, UK |
| <i>Δgmd</i> | <i>B. melitensis</i> 16M <i>Δgmd</i> | This study |
| <i>Δwada</i> | <i>B. melitensis</i> 16M <i>Δwada</i> | This study |
| <i>ΔOAg-lig</i> | <i>B. melitensis</i> 16M <i>ΔOAg-lig</i> | This study |
| <i>Δwada pBBR wada</i> | <i>B. melitensis</i> 16M <i>Δwada pBBR_MCSI_wada</i> | This study |
| <i>ΔOAg-lig pBBR OAg-lig</i> | <i>B. melitensis</i> 16M <i>ΔOAg-lig pBBR_MCSI_OAg-lig</i> | This study |
| <i>Δwada pBBR wada-R614A</i> | <i>B. melitensis</i> 16M <i>Δwada pBBR_MCSI_wada-R614A</i> | This study |

| <i>Agrobacterium tumefaciens</i> C58 | | | | |
|--------------------------------------|---------------------------------------------|------------|------|-------|
| WT | <i>A. tumefaciens</i> C58, Amp ^R | Strain | CFBP | #1903 |
| | | ATCC33970 | | |
| <i>mNG-lptC</i> | <i>A. tumefaciens</i> C58 <i>mNG-lptC</i> | This study | | |
| <i>mNG-lptF</i> | <i>A. tumefaciens</i> C58 <i>mNG-lptF</i> | This study | | |

Table S2 | List of primers used in this study. Nucleotides not annealing on DNA template are shown in bold, red characters represent point mutations and UPPERCASE corresponds to restriction site.

| Primer name | 5'-3' sequence |
|--------------------------------|-------------------------------------------------------------------------------------------|
| pNPTS138-rgsEmNG-F | gagcccgcg cagagctgacatctggatccacgaattcgc |
| pNPTS138-rgsEmNG-R | ttcagcaagttccttgagc gatcctgcagagaagcttggc |
| rgsEmNG-UP-F | gcc aagcttctctgcaggatcgctcaaggaactgctgaaattgt |
| rgsEmNG-UP-R | tcttcttcgcccttcgag accgaaggcgtccgcttgcac |
| mNG-4-rgsE-F | atgcaagcggagcgccttc gggtctcgaagggcgaagaaga |
| mNG-4-rgsE-R | ttttaactggagccgctcta cttatacagttcatccatgcc |
| rgsEmNG-DW-F | gcatggatgaactgtataa gtagagcggctccagttaaattgagca |
| rgsEmNG-DW-R | gcgaattcgtggatccagat gtcagctctgcgcggggtc |
| check-rgsEmNG-F | aagcgaaggaaacgacgac |
| check-rgsEmNG-R | acttgcaattgctgatccc |
| lptD_AGDP_UP_F_SpeI | ctagaACTAGT gaaatcaccacattcaacaatgg |
| lptD_AGDP-G4-3F-4_UP-R2 | cgatgtcatgatctttataatcaccgtcatgg tctttgtagtcgccaccgcccggg atcgcccgcaggtag |
| <i>lptD_AGDP-G4-3F-G4_DW-F</i> | ttataaagatcatgacatcgattacaaggatgacgatgacaagggcggcggtgg caatga aatgtattcaaagcagc |
| <i>lptD_AGDP_DW-R_SalI</i> | tgcacGTCGAC gccaaagctggaaggactg |
| mNlptC-UP-F | caggatgacacccttac |
| mNlptC-UP-R | tcatgg tctttgtagtcctatgtatcctttggcacatca |
| mNG-4-lptC-F | atggactacaaagaccatga |
| mNG-4-lptC-R | cggaacatgaatatgtgga |
| mNlptC-DW-F | cggtcggccaccggatcc acggccgacataccacat |
| mNlptC-DW-R | ttgttgcgaaagcgtg |
| check-mNlptC-F | aaaagggtcatgccag |
| check-mNlptC-R | tcgtcatgtctttatccc |
| mNlptF-UP-F | ggcagtcctgcccgaag |
| mNlptF-UP-R | gtcatgg tctttgtagtcctataggtggagtctacatccc |
| mNG-4-lptF-F | atggactacaaagaccatga |
| mNG-4-lptF-R | aaaTCTAGA cttatacagttcatccatgc |
| mNlptF-DW-F | aaaTCTAGAcggtcggccaccggatccc gattgatcgagttgtaca |
| mNlptF-DW-R | tttccatcagcagcatg |
| check-mNlptF-F | ttcctctctcatgtgaga |
| check-mNlptF-R | ccgaatcctgattcatcg |
| pNPTS-mNG-lptC-AT-F | gcgaagttccag tctcggaattcgctagcttcggccgtg |

| | |
|-------------------------------------|---------------------------------------------|
| pNPTS-mNG-lptC-AT-R | cgcttcgcgaggttatatgatcgtggatccagatatcctgc |
| mNlptC-AT-UP-F | caggatatctggatccacgatcatataacctgcggaagcg |
| mNlptC-AT-UP-R | tcttcgcccttcgagaccacaatcgaagcttggtctcta |
| mNG-4-lptC-AT-F | tagagacacaagcttcgattgtggtctcgaagggcgaaga |
| mNG-4-lptC-AT-R | cgggatccggtggccgaccgcttatacagttcatccatgccc |
| mNlptC-AT-DW-F | agcggtcggccaccggatcccgctgaatggcgccgggga |
| mNlptC-AT-DW-R | cacggccgaagctagcgaattccgcagactggaacttcgctgt |
| Check-mNlptC-AT-F | tcccttgacctctccag |
| Check-mNlptC-AT-R | cagaacacgaccagcagc |
| pNPTS-mNG-lptF-AT-F | tgccaaggaaggcatgatcgattcgctagcttcggccgtg |
| pNPTS-mNG-lptF-AT-R | gcggtgccccatttcaagaatcgtggatccagatatcctgc |
| mNlptF-AT-UP-F | caggatatctggatccacgattcttgaatgggcagccgc |
| mNlptF-AT-UP-R | tcttcgcccttcgagaccatacgccctgttcgatattgt |
| mNG-4-lptF-AT-F | acaatatcgaacagggccgtatggtctcgaagggcgaaga |
| mNG-4-lptF-AT-R | ttggatccggtggccgaccgcttatacagttcatccatgccc |
| mNlptF-AT-DW-F | agcggtcggccaccggatccaagcttctcgagaactatatcct |
| mNlptF-AT-DW-R | cacggccgaagctagcgaatcgatcatgccttccttgga |
| Check-mNlptF-AT-F | ccaattgccaacgatgcg |
| Check-mNlptF-AT-R | acgaaccagatggtcgtc |
| msbAmNG-UP-F | ttctatgacctcgaccgg |
| msbAmNG-UP-R | ggatccggtggccgaccgtctggcctctcctgtg |
| mNG-4-msbAm-F2 | cggtcggccaccggatccgactacaaagaccatgacg |
| mNG-4-msbAm-R2 | cacttcctcgctcacttatacagttcatccatgcc |
| msbAmNG-DW-F | tgagcgaggaagtgcaa |
| msbAmNG-DW-R | ggccagtatagccatcac |
| check-msbAmNG-F | tgcgcaacctctatctg |
| check-msbAmNG-R | ggccagtatagccatcac |
| pSKmsbAmNG-F | aaaACTAGTtgcgcaacctctatctg |
| pSKmsbAmNG-R | ttttCTAGAtcacttatacagttcatccatg |
| pSKmsbA _{E491A} -mNG-UP-F | aaaACTAGTtgcgcaacctctatctggg |
| pSKmsbA _{E491A} -mNG-UP-R | ttgtcgagcgcgacgttgctgcgtcgagaagcaggaccg |
| pSKmsbA _{E491A} -mNG-DW-F | tcgacgcagcaacgtcc |
| pSK msbA _{E491A} -mNG-DW-R | tttTCTAGAtcacttatacagttcatccatgcc |
| check-Δgmd-F | agctaacttgctggcataag |
| check-Δgmd R | caggtcgagtcgatcatg |
| ΔwaaL-UP-F | aattgcaatgcctataatgc |
| ΔwaaL-UP-R | ttcatgaagtccatccgatcagatatccaaaatgtgcc |
| ΔwaaL-DW-F | tcggagtgacttcatgaa |
| ΔwaaL-DW-R | cgccatatgcagatgttcg |
| ΔwadA-UP-F | gatcaagcaggtgctcatc |
| ΔwadA-UP-R | tgtaaaagtggaaccgctctaattcgcttgccctcagc |

| | |
|----------------------------|------------------------------------------------------|
| ΔwadA-DW-F | gagcggttccacttttaca |
| ΔwadA-DW-R | gcaaatatcagcgcaacg |
| check-ΔwadA-F | gaaacggtgCGGataatc |
| check-ΔwadA-R | aacgcataatcgaagcttt |
| ΔOAg-lig-UP-F | cgaggttcgcatatatctatc |
| ΔOAg-lig-UP-R | taacaattcccaggatgtatt |
| ΔOAg-lig-DW-F | aatacatcctgggaattgttaattcgctatggatttctgg |
| ΔOAg-lig-DW-R | gaagtgcctggcaaacttt |
| check-ΔOAg-lig-F | gaaacggtgCGGataatc |
| check-ΔOAg-lig-R | aacgcataatcgaagcttt |
| pBBRI-wadAc-F | tttCTGCAGatttctcgctcgatcgc |
| pBBRI-wadAc-R | tttCTCGAGattacaggcttgtcaggg |
| pBBRI-OAg-lig-UP-F | ttttGAGCTCatttctcgctcgatcgc |
| pBBRI-OAg-lig-UP-R | aaaaCATATGcattaattcgcttgcctca |
| pBBRI-OAg-lig-DW-F | aaaCATATGtccgatcagaattcaggtt |
| pBBRI-OAg-lig-DW-R | aaaaCTGCAGattacaggcttgtcaggg |
| pBBRwadA-R614A-UP-F | tttCTGCAGatttctcgctcgatcgc |
| pBBRwadA-R614A-UP-R | cataggcaatggcaatttcag |
| pBBRwadA-R614A-DW-F | ctggaaattgccattgctatggatttctgggcttgcgtg |
| pBBRwadA-R614A-DW-F | tttCTCGAGattacaggcttgtcaggg |
| mNG-wadA-UP-F | tcaaccttctctccaatgc |
| mNG-wadA-UP-R | cgtcatggtctttgtagtcattaattcgcttgcctc |
| mNG4wadA-F | gactacaaagaccatgacg |
| mNG4wadA-R | ggatccggtggccgaccgcttatacagttcatccatgc |
| mNG-wadA-DW-F | cggtcggccaccggatccatattgcccgtattcgtca |
| mNG-wadA-DW-R | tttgagatttcgctgtgag |
| wadA-mNG-UP-F | aaaagccagacctcacg |
| wadA-mNG-UP-R | ggatccggtggccgaccggaagtaggtgcgcggg |
| mNG4wadAm-F | cggtcggccaccggatccgactacaaagaccatgacggt |
| mNG4wadAm-R | taaaagtggaaaccgctcttacttatacagttcatccatgcc |
| wadA-mNG-DW-F | taagagcggttccactttta |
| wadA-mNG-DW-R | acctgggaacatatggcg |
| Check-wadA-F | gatcaagcaggtgctcatc |
| Check-wadA-R | gcaaatatcagcgcaacg |
| pNPTS138_mNG-wzm-F | gagccatttttacctatgaatctggatccacgaattcgc |
| pNPTS138_mNG-wzm-R | cgatttttcgtcacaaatctatcctgcagagaagcttggc |
| mNG-wzm-UP-F | gccaagcttctctgcaggatagatttgtgacgaaaaatcgc |
| mNG-wzm-UP-R | tcttcttcgcccttcgagaccatacgaaatcgtctcactaaa |
| mNG4wzm-F | tagtgagacgatttcgtatggtctcgaagggcgaagaaga |
| mNG4wzm-R | acattagccatatacgatatcttatacagttcatccatgcc |
| mNG-wzm-DW-F | gcatggatgaactgtataagatatcgtatatggctaattgtctgg |

| | |
|----------------------|---------------------------------------------------|
| mNG-wzm-DW-R | gcgaattcgtggatccagattcataggtaaaaaatggctctc |
| Check-mNG-wzm | tgtgacatggatcgatcc |
| Check-mNG-wzm | cagggtaatcgatggctg |

Table S3 | List of plasmids used in this study.

| Plasmid name | Reference |
|----------------------------------------------|---------------------------------------------------------|
| pNPTS138 | M. R. K. Alley, Imperial College of Science, London, UK |
| pSKoriT kan | 2 |
| pKSoriT cat | Isabelle Danese and Pascal Lestrade, UNamur, BE |
| pBBR_MCSI | 3 |
| pNPTS138_rgsE-mNG | This study |
| pNPTS138_lptD::lptD-3Flag | V. Vassen, PhD thesis, UNamur, BE |
| pNPTS138_mNG-lptC | This study |
| pNPTS138_mNG-lptF | This study |
| pNPTS138_msbA-mNG | This study |
| pSKoriT_kan_pdhS-mCherry | 4 |
| pKSoriT_cat_popZ-mCherry | 5 |
| pSKoriT_kan-msbA-mNG | This study |
| pSKoriT_kan- msbA_{E491A}-mNG | This study |
| pNPTS138_mNG-lptC-AT | This study |
| pNPTS138_mNG-lptF-AT | This study |
| pNPTS138_ΔwaaL | This study |
| pNPTS138_ΔwadA | This study |
| pNPTS138_ΔOAg-lig | This study |
| pBBR_MCSI_waaL | This study |
| pBBR_MCSI_wadA | This study |
| pBBR_MCSI_OAg-lig | This study |
| pBBR_MCSI_wadA-R614A | This study |
| pBBRI_P_{lacZ}_gmd | 1 |
| pNPTS138_Δgmd | 1 |
| pNPTS138_mNG_wadA | This study |
| pNPTS138_wadA_mNG | This study |
| pNPTS138_mNG_wzm | This study |

Table S4 | List of primary antibodies used in this study.

| Name | Recognized structure | Host (Isotype) | Application | Reference |
|------------------|-----------------------------------|----------------|---------------------|-----------|
| A76/12G12 | <i>Brucella</i> S-LPS (O-antigen) | Mouse (IgG1) | WB, IF | 6 |
| B66/04F09 | <i>Brucella</i> S-LPS (O-antigen) | Mouse (IgG2a) | IF, S-LPS induction | 7 |

| | | | | |
|----------------------------|----------------------------------------------------|---------------------|-------------|---------------------------------------|
| A68/24D08/G09 | <i>Brucella</i> R-LPS (lateral branch of the core) | Mouse (Unknown) | WB | ⁸ |
| Anti-LptD | <i>Brucella</i> LptD | Rabbit (Polyclonal) | WB | This study ThermoFisher Scientific |
| DYKDDDDK Tag (FG4R) | 3Flag tag | Mouse (IgG2b) | WB, IF- SEM | |
| A68/7G11/C10 | <i>Brucella</i> Omp10 | Mouse (IgG2a) | WB | |

Table S5 | List of secondary antibodies used in this study.

| Target | Conjugate | Application | Reference |
|------------------------|-----------------|-------------|----------------------|
| Mouse IgG (H+L) | Alexa Fluor 514 | IF | Invitrogen (A-31555) |
| Mouse | HRP | WB | Dako (P0260) |
| Rabbit | HRP | WB | Dako (P0217) |
| Mouse | 18 nm gold | IF-SEM | Abcam |

Table S6 | Table of ORF investigated in this study.

| Gene name | ORF number in <i>B. abortus</i> 2308 | UniProt accession number | NCBI Hyperlink |
|-------------|--------------------------------------|--------------------------|-----------------------------------------------------------------------------------------------------------------|
| <i>rgsE</i> | BAB1_1043 | Q2YQ47 | https://www.ncbi.nlm.nih.gov/protein/CAJ10999.1/ |
| <i>lptD</i> | BAB1_0707 | Q2YN48 | https://www.ncbi.nlm.nih.gov/protein/CAJ10663.1/ |
| <i>lptC</i> | BAB1_0154 | Q2YP16 | https://www.ncbi.nlm.nih.gov/protein/CAJ10110.1/ |
| <i>lptF</i> | BAB1_0709 | Q2YN46 | https://www.ncbi.nlm.nih.gov/protein/CAJ10665.1/ |
| <i>msbA</i> | BAB2_1011 | Q2YJN8 | https://www.ncbi.nlm.nih.gov/protein/CAJ13177.1/ |
| <i>gmd</i> | BAB1_0545 | Q2YMP3 | https://www.ncbi.nlm.nih.gov/protein/CAJ10501.1/ |
| <i>waal</i> | BAB2_0106 | Q2YJ92 | https://www.ncbi.nlm.nih.gov/protein/CAJ12272.1/ |
| <i>wadA</i> | BAB1_0639 | Q2YMX2 | https://www.ncbi.nlm.nih.gov/protein/CAJ10595.1/ |
| <i>wzm</i> | BAB1_0543 | Q2YMP5 | https://www.ncbi.nlm.nih.gov/protein/CAJ10499.1/ |
| Gene name | ORF in <i>A. tumefaciens</i> C58 | UniProt accession number | NCBI Hyperlink |
| <i>lptC</i> | Atu0335 | Q7D1N8 | https://www.ncbi.nlm.nih.gov/protein/15155241 |
| <i>lptF</i> | Atu1108 | A9CJH0 | https://www.ncbi.nlm.nih.gov/protein/15156140 |

Table S7 | Table representing the percentage of S-LPS and LptCF protein colocalization upon S-LPS induction. Only the bacteria displaying focus for LptC/F and labelling for S-LPS were taken into account for the analysis. When S-LPS signal was detected at one pole, LptC and LptF were also detected at the same pole in 67.18 and 77.70% of the case. In 9.06 and 2.59% of bacteria for mNG-lptC or mNG-lptF respectively, no signal was detected for S-LPS when a focus could be observed at the mid cell either for LptC or LptF. This could be due to a low amount of newly incorporated S-LPS at the moment of the labelling that would be below the detection threshold. In 7.86 and 4.60% of the time, LptC and LptF were observed at both poles of the bacteria when the S-LPS signal was only at one pole. LptC and LptF were sometimes (in 3.76 and 6.61% respectively) localized at the opposite pole to the one labelled for S-LPS. Mid cell localisation was also observed in 1.88% for LptC and 2.30% for LptF for bacteria only labelled at one pole for S-LPS. When the S-LPS signal was also detected at the new pole and the constriction site, LptC and LptF foci could be either detected at the same area as S-LPS (4.79% and 2.73% respectively), or at the mid cell only (4.96 and 5.47% respectively). Lastly, S-LPS signal was sometimes observed at the mid cell only, and in that case, 0.51% of the bacteria also displayed a focus for LptC, and 1% of the bacteria displayed a focus for LptF. When the signals are not colocalizing, the percentage is represented in grey. Although not represented here, it should be stated that after 9h of IPTG induction, only 20% and 16% of the whole bacterial population were labelled for S-LPS incorporation in *mNG-lptC* and *mNG-lptF*, as opposed to about 41% for the control (*Δgmd* pBBRi *gmd*). Therefore, one should note that even if the fusions (*mNG-lptC* and *mNG-lptF*) are viable, the functionality of the fusion proteins might slightly impair the efficacy of LPS export to the OM.

| | % of LptC foci | % of LptF foci |
|-----------------------------------------------------|----------------|----------------|
| S-LPS signal at one pole | | |
| Lpt protein at the same pole | 67.18 | 77.70 |
| Lpt protein at the same pole & at mid cell | 9.06 | 2.59 |
| Lpt protein bipolar | 7.86 | 4.61 |
| Lpt protein opposite pole | 3.76 | 3.60 |
| Lpt protein at mid cell | 1.88 | 2.30 |
| S-LPS signal at one pole and at the mid cell | | |
| Lpt protein at same pole & at mid cell | 4.79 | 2.73 |
| Lpt protein at mid cell | 4.96 | 5.47 |
| S-LPS signal at mid cell | | |
| Lpt protein at mid cell | 0.51 | 1.00 |
| Total | 100 | 100 |

| | | |
|---------------------------|----------------------------------------------------------------|-----|
| Bradyrhizobium_spp. | -----MPTLALGVLSA-----IDIA-TTAPALAQGFYTNPRPPHPAPPR | 39 |
| Brucella_abortus_544 | VGLSNTRMVLPHLTLRLARGTALACVLAL-PFVSVAIILSSPAQAQDAL-----SA--NYQ | 52 |
| Ochrobactrum_anthropi | -----MVLPHSLRLARGTALACVLAL-PFISVAALPSPAQAQDAL-----AS--NYE | 45 |
| Agrobacterium_tumefaciens | MAVF--DRGNIIRRLAALALTGASV--CAYVATASAFAD--DQVI-----VGD | 42 |
| Rhizobium_freirei | MAAG--NRKISRKIVAAALVTGTAA--CAYIMSPVWYAYQ--AHNNNGSTGT--VTSPIKVN | 53 |
| Sinorhizobium_fredii | MAAG--NRGPVKLTNAALLAGVAL--HVLIGMH-----APAMAQDTT--RIDEIQPN | 48 |
| Bradyrhizobium_spp. | VAHDNQMLVQANEVDYDYNHSRVSAGVNVQLFYNGTSVEADKVTYDQKTRLHAEGNIRM | 99 |
| Brucella_abortus_544 | SDPNARMLLQADELYVDYDNTVTAGQKVRIEYDGNRLVADKVTYDQKTRMTATGNIVEI | 112 |
| Ochrobactrum_anthropi | SDPNARMLLQADELYVDYDNTVTAGQKVRIEYDGNRLVADKVTYDQKTRMTATGNIVEI | 105 |
| Agrobacterium_tumefaciens | AQDQSKLLLTANELTYNRDAQEVVATGGVRLKYSGYRMVAQRIEYNGQTRGVVARGNIEL | 102 |
| Rhizobium_freirei | VPEQSKLLVSSNELTYNRDAQIVTATGAQVINYGGYRMVAQVIEYNGQSGRMALGNVEL | 113 |
| Sinorhizobium_fredii | IPADAKLLLTANELVYNRDTEIVTVRGVNIIEYGGYRMVAQVINYNGQSGRIILATGEVQL | 108 |
| Bradyrhizobium_spp. | TDAEKGITYANIMDLSDDYRDGFVDSLHVDATADTRMAATRVRSNGYTVFENGVTAC | 159 |
| Brucella_abortus_544 | VERDGNRIYSDHIDVDTDFRDGFVNLRVETTDNTRFAESAERSNGEITTFNNGVYATC | 172 |
| Ochrobactrum_anthropi | VERDGNRIYSEHMDVDTDFRDGFVNLRVETTDNTRFAESAERSNGEITTFNNGVYATC | 165 |
| Agrobacterium_tumefaciens | IEPSGNRIYADELDVDTDFRDGFVNLRVETTDNTRFAESAERSNGEITTFNNGVYATC | 162 |
| Rhizobium_freirei | ITPDGNRMYGDKMDVDTDFRDGFVNLRVEMPDNTRMAEGERVGGTGLILTKGVYATC | 173 |
| Sinorhizobium_fredii | IEPGGNIVYADKMDVDTDFRDGFVNLRIETDTRLAAESGERNGEITFLINKAVYATC | 168 |
| Bradyrhizobium_spp. | APCQDQPKKPLWQKVGARIIDHQVDRMLYFETAQLEFFGVPMAYLPFFSTPDPTVKRKS | 219 |
| Brucella_abortus_544 | EPCAKNPDKPVLWQIKARKIINWSATKTVRFERGRFELFGMPLAYLPAFEMADPTVKRKS | 232 |
| Ochrobactrum_anthropi | EPCAKNPDKPVLWQIKARKIINWSATKTVRFERGRFELFGMPLAYLPAFEMADPTVKRKS | 225 |
| Agrobacterium_tumefaciens | LPCAERPKPPLWQKVAERTVQDQKSGQVRLKARFELFGKPIAYLPFLTPDNRVKRKS | 222 |
| Rhizobium_freirei | KACSEQG--RAPLHQVKAQRVITQNGVTHTVRLHARFELFGQPIAYLPWIEVPDPTVKRKS | 232 |
| Sinorhizobium_fredii | TPCSKPEHRSWLHIKAQRVQNGRTRTIRLEHAYFELFGKPIAYLPAMEIPDPTVKRKS | 228 |
| Bradyrhizobium_spp. | GFLLMPGFSEASTYGFGEIPYWAIAPODYDATFSPIRITSTQGVLLQAEFRQLMDGSYQI | 279 |
| Brucella_abortus_544 | GFLLPFGFAYKDDLFGGINKSYFWALAPNYDLTLSTTAYTKQGLTEAENHRLLENGEYDF | 292 |
| Ochrobactrum_anthropi | GFLLPFGFAYKDDLFGGINKSYFWALAPNYDLTLSTTAYTKQGLTEAENHRLLENGTYNL | 285 |
| Agrobacterium_tumefaciens | GFLLPQMSITDKLFGGIGVPPYQVLGDSADLTVPFTFYTAQGLLHGLERNRFEHMTHTL | 282 |
| Rhizobium_freirei | GFLLPQMSITDKLFGGIGVPPYQVLGDSADLTVPFTFYTAQGLLHGLERNRFEHMTHTL | 292 |
| Sinorhizobium_fredii | GFLLPPTFRYAQKLGAQVGPYWAISPYMDATVATGTLTRQGLLEGEFRQRFHGWHITL | 288 |
| Bradyrhizobium_spp. | RAYGIDQLDPGAFAGGPGDR--QFRGGIETKQGFANDKMMWGWGVLSDFYFMQDYRL | 337 |
| Brucella_abortus_544 | RIAGTHQLKPEEFQVATIDREKTRNGMVASKGFNDITRWFHGMVDLQDTHMFSTRYIEI | 352 |
| Ochrobactrum_anthropi | RIAGTHQNKPGFQVNTVDREEDNRGMVASKGFNLNWRFRGMDVMAQDTRMFSTRYISL | 345 |
| Agrobacterium_tumefaciens | TFAGIDQDRPERDAGTSDALNDRRGVVASRGDFKINRWSFGMDVMQSDNNIFARTYDL | 342 |
| Rhizobium_freirei | RIAGIDQNMISGKFNAGVSDAEDHLRGMVSKADFIRNPRWTFGMDVMQSDNNIFSTRYGL | 352 |
| Sinorhizobium_fredii | NVAGISQMDRDQFTPGTDAEETGRGMVASKGRFENRPRWTFGMDVMQSDNNIFAKTYDL | 348 |
| Bradyrhizobium_spp. | SQYRDPMSFNLNLTPEATSLYLTVGERSFFDLRSIYLSF-----SGNQGVPIV | 389 |
| Brucella_abortus_544 | QGYNAQ-----TQVSKIYLTGINNRHYDLNRYFNQVQSY(AGDP)NEMYSKQPVW | 403 |
| Ochrobactrum_anthropi | DGYGAE-----TQVSKIYLTGINNRHYDLNRYFNQVQSELSDNPNEMYSKQPVW | 396 |
| Agrobacterium_tumefaciens | KGYKSE-----TQTHNKLYLTGLGRNHFDMANAYFNVQDK--DDFELEERRQATV | 390 |
| Rhizobium_freirei | EGVWQS-----THTNQAYLTGLGRNHFDMANAYFNIQDA--DHISITAQKQQAIV | 480 |
| Sinorhizobium_fredii | SSFDGT-----TYNQAYLTGLGRNHFDMANAYFYDQDA--DPHSIAEQQ-PI | 395 |
| Bradyrhizobium_spp. | YPLVDYSNIFHYPILGGEVSYKTNFVNLTDRDAVDPTITTLANTFGLCTLTSADPLARTP | 449 |
| Brucella_abortus_544 | FPSLDYSYTMPEPVYGGSELNFANLQALRYKNADYTNFISVDENGSWT-----KPN | 456 |
| Ochrobactrum_anthropi | FPSLDYSYTMPEPVYGGSELNFANLQALRYKNADYTNFISVDENGSWT-----KPN | 445 |
| Agrobacterium_tumefaciens | HPAVDYRYFLPDYVYGGSELNLTSLTRRNQDAYA--L-----DSS | 429 |
| Rhizobium_freirei | YPSIDYHYVDPKSVLGGELSATMNFTHLSRDKTSVLDATTALG-----DSS | 446 |
| Sinorhizobium_fredii | AQVLDYSYTAPEVPLGGELNADFNFTNVRNRLDRD--TTV-----F | 435 |
| Bradyrhizobium_spp. | TQCLLRGPGTYTRFATAEQWRRSFTDPAGEIWTPTATVRADAINSDVSNQGVAMFL-P | 508 |
| Brucella_abortus_544 | PYPNPGFSGTNLRFTEAEWKRTFITPSGLVITPLALRGDAIRVDTHFDPAMAGTDA | 516 |
| Ochrobactrum_anthropi | RYPRVPGFDGTNARLTTEAEWKRTFITPAGLVITPLALRGDAIGANTNFNLADAGYTD | 505 |
| Agrobacterium_tumefaciens | TSNRFPGLEGTYTRLTTEAEWKRTYTFDSGLQLTPLGALRGDVFSTOMATQGL--TYGSS | 487 |
| Rhizobium_freirei | LNDRYLKLGQDYRLSTELQWQRTFTDQGLVTLPLAARGDIYGLDMNAPGAGTYSGNY | 506 |
| Sinorhizobium_fredii | GVDRFRGLESGSHRLTGELEWKKFTIVPGGLALTPLAARGDAVGIVNDP--IGYTGFE | 493 |
| Bradyrhizobium_spp. | VGDEALRMVPTVGLERYRPIINVQWGSTTIEPIAQIIRPNETIYAGKLPNEDAQSUVF | 568 |
| Brucella_abortus_544 | VVRSEALRMVPTAGLELRWPLFTSTTHILEPVAQIFVRNRIYAGQLPNEDAQSUVF | 576 |
| Ochrobactrum_anthropi | LVRSEALRAMATAGLELRWPLFTSTTHILEPVAQIFVRNRIYAGLEPNEDAQSUVF | 565 |
| Agrobacterium_tumefaciens | IDDDAARFMATLGLLEARYPILFTAQHSHVIEPIAQVVRPDEQFAGRLPNEDAQSUVF | 547 |
| Rhizobium_freirei | DNSDYPTRGMITAGLEARYPILFTTNHSHVIEPIAQVVRPDEQFAGRLPNEDAQSUVF | 566 |
| Sinorhizobium_fredii | NSSDAVTRGMITAGLEARYPILFAGETSSHVLEPIAQVYARPNQYAGALPNEDAQSUVF | 553 |
| Bradyrhizobium_spp. | DTSNLFSDIKFSGYDRVEGGGRANVGQVTTQFDH--GGTYKALFGQSYQLFGLNSFAVRD | 627 |
| Brucella_abortus_544 | DASNLFSDIKFSGYDRVEGGGRANLGLRYSNGFNDSNIALYALGQSFQGLGGLNSYAASD | 636 |
| Ochrobactrum_anthropi | DATNLFSDIKFSGYDRVEGGGRANLGLRYSNGFNDSNIALYALGQSFQGLGGLNSYGTSD | 625 |
| Agrobacterium_tumefaciens | DATNLFERDKFTGDFRVEGGGRANLGLRYSNGFTDN--GYGIRATAGSFLHAGENSFASPD | 606 |
| Rhizobium_freirei | DATNLFDRDKFSGDFRVEGGGRANVWRYTGSFON--GYKLQHTFGQSYQLGGRNSFATD | 625 |
| Sinorhizobium_fredii | DATNLFDRDKFSGDFRVEGGGRANVGIYRTGSFDS--GYGLRATAGSFLHAGENSFATD | 612 |
| Bradyrhizobium_spp. | EINTGVDSGLQNARSQVYASVSDYSPNRTYTFSVRSRDEQTLVQRFEEAERANFRDWSV | 687 |
| Brucella_abortus_544 | FVWNGADSGLEADRSQVYAMIGTSNSTGLVLAARGRFQKDFAVQRFEEAQSQWEKLT | 696 |
| Ochrobactrum_anthropi | FVWNGADSGLEADRSQVYAMIGTSNSTGLAARGRFQKDFAVQRFEEAQSQWEKLT | 685 |
| Agrobacterium_tumefaciens | LVMAGADSGLEADRSQVYMAADAPIGLSFNSRLRDKDNFIEINRMENSVHYTDNRFTG | 666 |
| Rhizobium_freirei | LAVGSDSGLETTSDYVTMFGTLTPQGISLATSRYRDEKDFAFRRGDTSVGFSNIDFQT | 685 |
| Sinorhizobium_fredii | LKVAGADSGLETTSDYVAMVGDVAPSGMASLGRLEKDLDFRRADATVGYLGLTWQA | 672 |
| Bradyrhizobium_spp. | SLIYGNYAQPDLGYLTRREGLLGSGSVKVTANWVSGAARNDLEANINQYITIGAYVD | 747 |
| Brucella_abortus_544 | SGQYAYIAPQAYGYSDLRQEVGTGSATARINTNRRVFGSGTYDLSOTLVRASSGLAYDD | 756 |
| Ochrobactrum_anthropi | SAQYAYIAPQAYGYSDLRQETGTSATARINTNRRVFGSGTYDMSVETLVRASSGLAYDD | 745 |
| Agrobacterium_tumefaciens | KVSYTQVKAQPNYGYDRDRDIQTSGKIRLDNNMALGATINYLNNKFSERRIGVLQD | 726 |
| Rhizobium_freirei | SLIYTHIAAQPYGFTSNQDEIQSRAQIKFKEYNSVFGTSYSDINSQDFTROQVGLSYED | 745 |
| Sinorhizobium_fredii | AFYTRIEAQLYGSQSDQDEIQTAAAYRFHDFNSVFGALTYDINNQVSRHGVGLTYDD | 732 |
| Bradyrhizobium_spp. | DCFVLAANYVTSFNYATPTTPPVLSHAYMLQIGLRLANTSSSSGPMGVQ----- | 797 |
| Brucella_abortus_544 | ECFTYSMAYIQTNRPGD-----EKASHVGFITISRLTLDGFGNGSQTFNGLAKRKGKYG | 812 |
| Ochrobactrum_anthropi | ECFTYSMAYIQTNRPGD-----DKASHVGFITISRLTLDGIGSGNQTF----- | 788 |
| Agrobacterium_tumefaciens | ECTFTYSYSDGEGNLSVTRF--AANDWSINARLAFRTLGDISVGSAAEDQK--ADIGWQPN | 784 |
| Rhizobium_freirei | ECTFTYSVFLNKD--STAQ--AANDWSVGARLFTRLGDVNLGNVQIATF----- | 792 |
| Sinorhizobium_fredii | QDTLFSIVKSERD--TOST--VANDWSIGARLFTRLGDINNGVTRFEEL-----DYF-- | 782 |
| Bradyrhizobium_spp. | ----- | 797 |
| Brucella_abortus_544 | QCIRHFLFAQGSAPACHKPVFSWSGSLTETA | 845 |
| Ochrobactrum_anthropi | ----- | 788 |
| Agrobacterium_tumefaciens | NY----- | 786 |
| Rhizobium_freirei | ----- | 792 |
| Sinorhizobium_fredii | ----- | 782 |

Figure S1 | Alignment of Rhizobiales LptD amino acid sequences. Sequences of LptD homologs in other Rhizobiales were identified by Blast-P¹⁰ and multiple sequence alignment was generated with CLUSTAL Omega (1.2.4)¹¹. Position of the insertion of a 3Flag tag is shown with a green box (AGDP loop, position 383 in *B. abortus* 544 sequence). The ADGP sequence was duplicated and a Gly linker sequence was inserted on each side of the 3Flag.

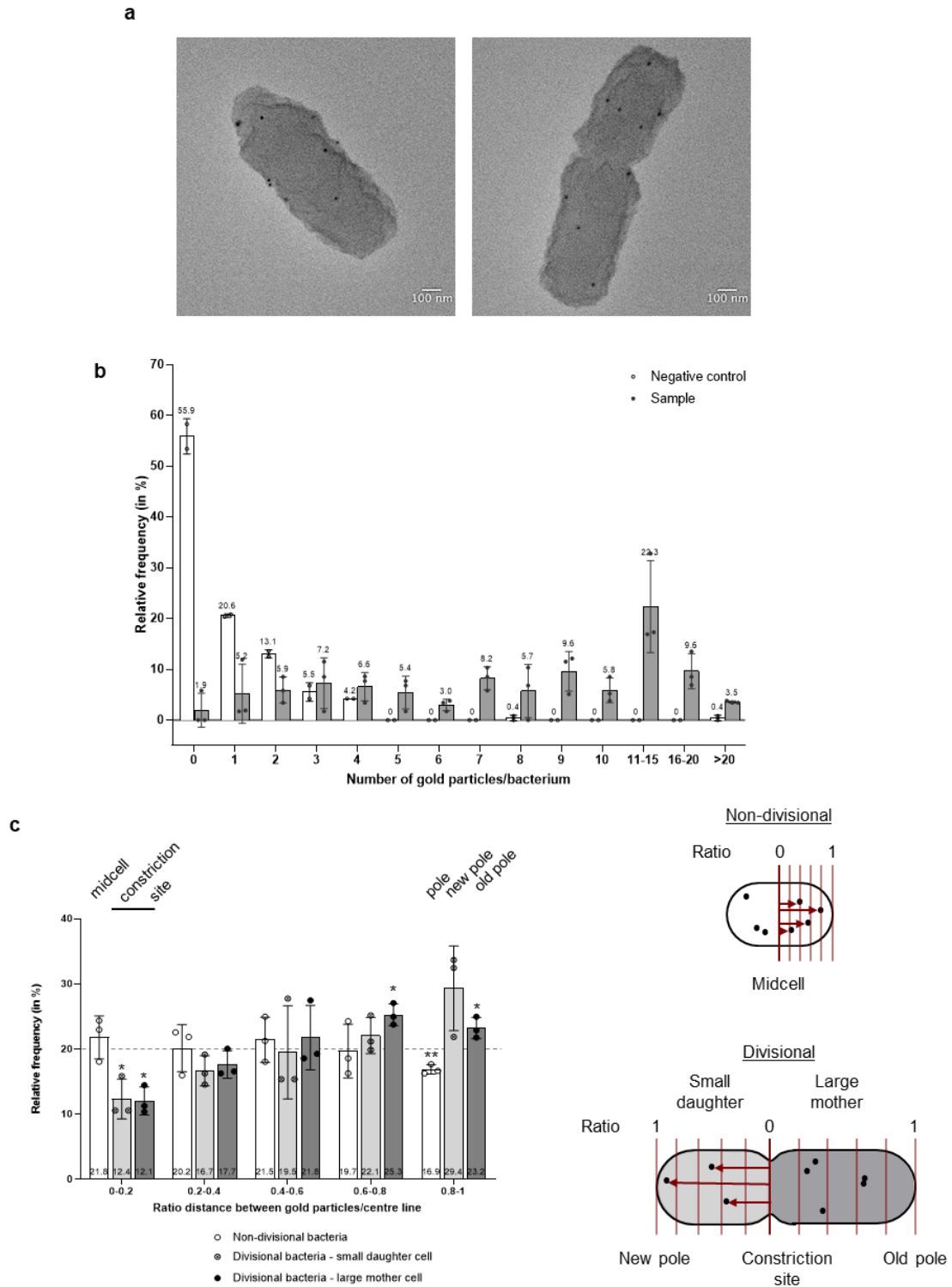


Figure S2 | Localization of LptD. a. SEM images from exponential phase culture of *B. abortus* WT and *3Flag::lptD*. Scale bar is 100 nm. Images were acquired by low angle backscattered electron (LAFE) mode and inverted. Black dots correspond to gold particles. Brightness (+20%) and sharpness (+50%) of images were adjusted (n=3). **b.** Frequency distribution of gold particles from negative control (*B. abortus* disrupt *gmd*) and sample (*B. abortus* 544 disrupt *gmd lptD-3Flag*) after IF-SEM. Data are presented as mean values (corresponding numbers are shown on top of the bars) and error bars correspond to standard deviation from two and three independent experiments, respectively. $n_{\text{negative control}}=142$ bacteria, $n_{\text{sample}}=169$ bacteria. Source data are provided as a Source Data file. **c.** The distribution of gold particles labelling *3Flag::LptD* was analyzed by measuring the distance towards central line in non-divisive and towards constriction site in divisive bacteria. Ratios of distance of gold particles/central line were classified in five categories of equal size (model on right side, ratio distances as shown as red arrows). Divisive bacteria were further divided in daughter and mother part according to their size. Relative frequencies are shown as mean values (corresponding numbers are shown at the bottom of each bar) and error bars correspond to standard deviation from three independent experiments. Grey dashed line represents theoretical frequency of random distribution (20%). Statistical differences compared to a theoretical relative frequency of 20% were analyzed by one-sided t-test. * $p<0.05$ (0.025 for small daughter (ratio of 0-0.2), 0.012 for large mother cell (ratio 0-0.2), 0.016 for large mother cell (ratio 0.6-0.8), and 0.016 for large bacteria (ratio 0.8-1)), ** $p<0.01$ (non-divisive bacteria (ratio 0.8-1)). Number of non-divisive bacteria= 58, Number of divisive bacteria=77. Source data are provided as a Source Data file.

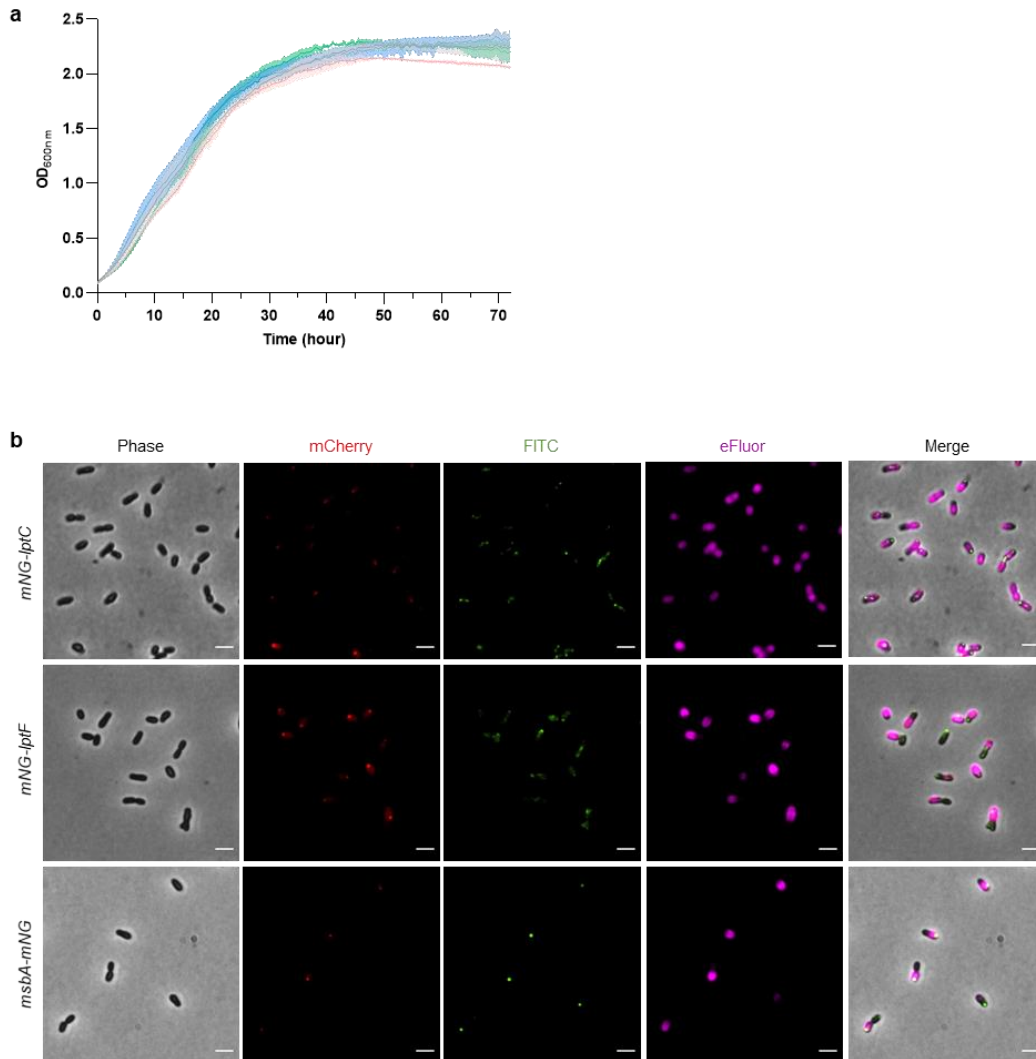


Figure S3 | Growth behaviour of *mNG-lptC*, *mNG-lptF* and *msbA-mNG* strains. **a**, Exponential phase cultures of WT (grey), *mNG-lptC* (pink), *mNG-lptF* (blue) and *msbA-mNG* (green) were diluted to OD₆₀₀ = 0.1 and grown in TSB rich medium at 37°C for 72 h. The OD₆₀₀ was measured every 30 minutes. Dotted lines correspond to one standard deviation and plain line corresponds to the mean of the biological triplicates. N=3. Source data are provided as a Source Data file. **b**, eFluor labelling of *mNG-lptC*, *mNG-lptF* and *msbA-mNG* was performed on early exponential phase culture in strains co-expressing PdhS-mCherry. Bacteria were labelled with eFluor, washed and grown for 2.5 h at 37°C. The eFluor670 (eFluor) covalently binds to the amine groups on the bacterial cell surface, therefore the labelled part corresponds to the old envelope material and the unlabelled part corresponds to newly incorporated material. mNG corresponds to the channel of the fusion with the fluorescent protein mNG. The experiment was performed in biological triplicate for each strain (n=3). Scale bars are 2 μ m. Source data are provided as a Source Data file.

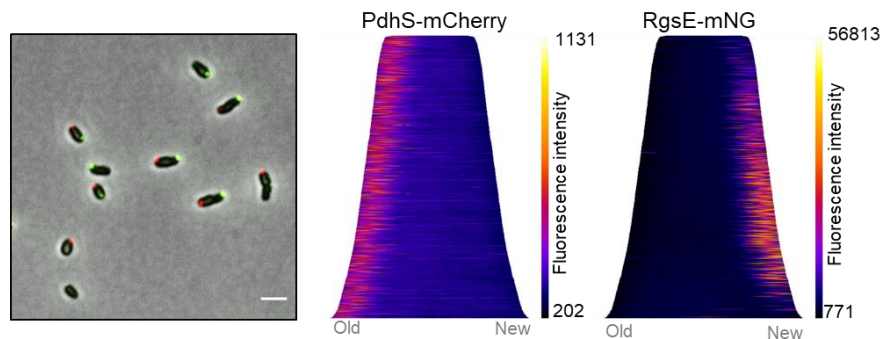


Figure S4 | Polar localization of Rhizobiales growth and septation protein RgsE is conserved in *B. abortus*. Representative merged picture with phase and fluorescent channels of the strain co-expressing PdhS-mCherry (red) and RgsE-mNG (green). The scale bar is 2 μ m. The right panels show demographic representations of PdhS-mCherry and of RgsE-mNG localization. Cells are aligned based on their length (smallest on top) and pole age using PdhS-mCherry as an old pole marker (old pole on the left). The graphs correspond to one representative experiment (n=1482 bacteria). Fluorescence intensity is represented as a heatmap, the minimum and maximum values represented on the scale were automatically selected to provide the best signal to background by MicrobeJ. Source data are provided as a Source Data file.

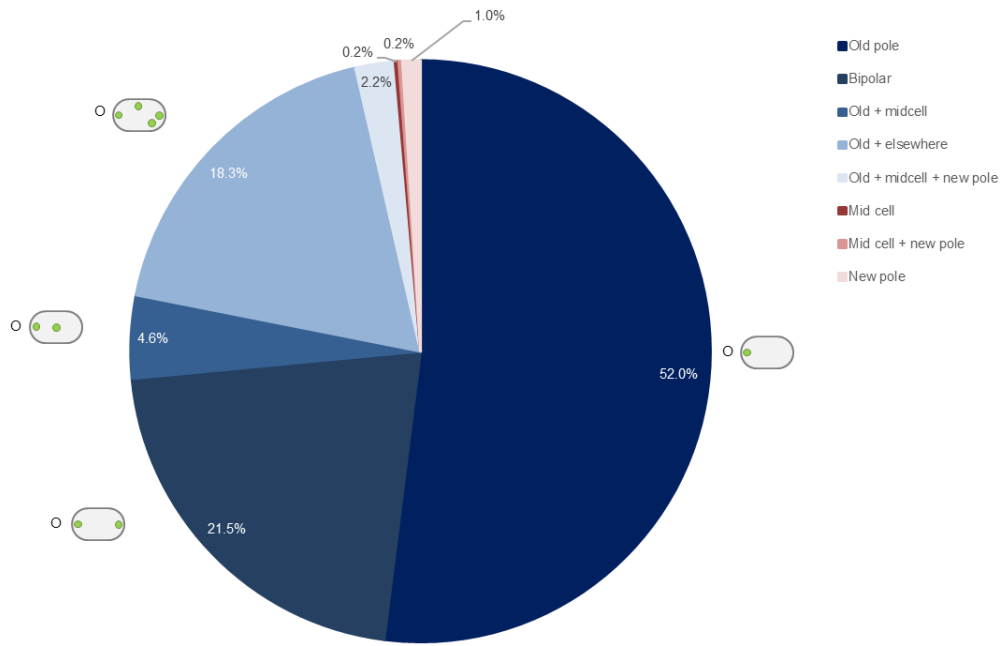


Figure S5 | MsbA localization in *B. abortus*. MsbA is found at the old pole in 98.6% of bacteria. 52% of the cells display only one focus of MsbA at the old pole, 21.5% show a bipolar localization of MsbA, 4.6% display one focus at the mid cell and the old pole, and 18.3% show one focus of MsbA and at least another somewhere else in the bacterium. 2.2% percent of the bacteria display one focus at the old pole, mid cell and at the new pole. Only 0.2% had a focus at the mid cell, 0.2% at the new pole and the division site and 1% had one focus at the new pole only. The analysis was performed by manual counting of bacteria of the strain co-expressing MsbA-mNG and PdhS-mCherry labelled with eFluor. O: Old pole. N=540 bacteria for two biological replicates. Source data are provided as a Source Data file.

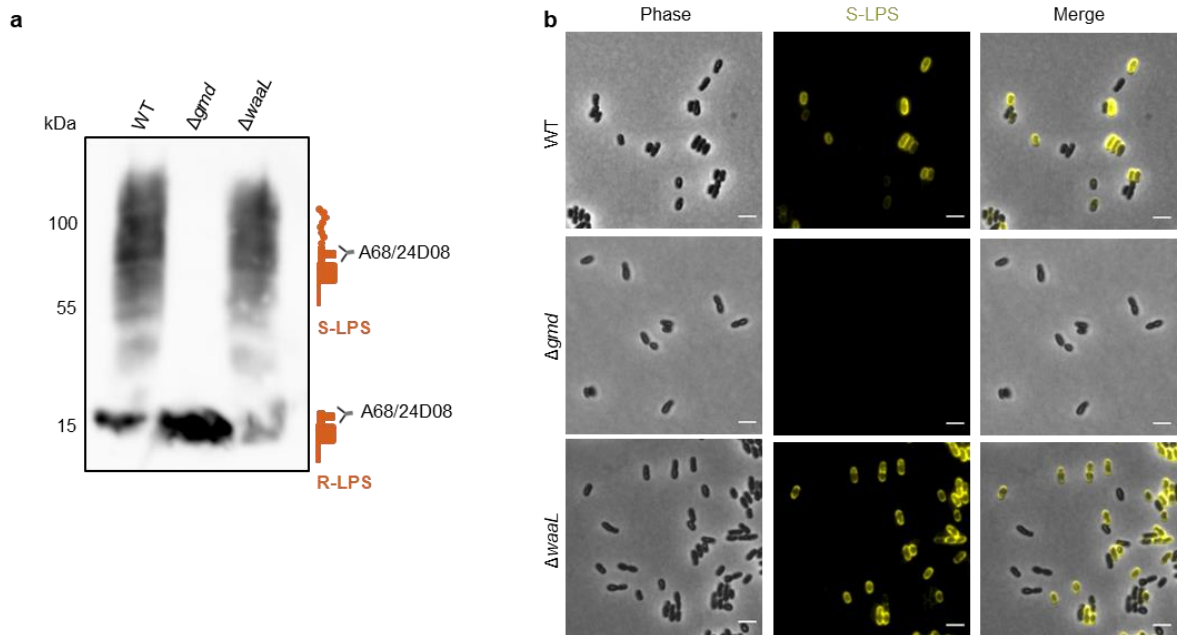


Figure S6 | S-LPS phenotype of *B. abortus* $\Delta waaL$. **a**, Western blot analysis performed using the A68/24D08 mAb targeting the core of both R-LPS and S-LPS (n=3). Source data are provided as a Source Data file. **b**, Immunofluorescence microscopy labelling S-LPS of WT, Δgmd and $\Delta waaL$ using the mAb A76/12G12. The experiment was performed in biological triplicate (n=3). Scale bars are 2 μm . Source data are provided as a Source Data file.

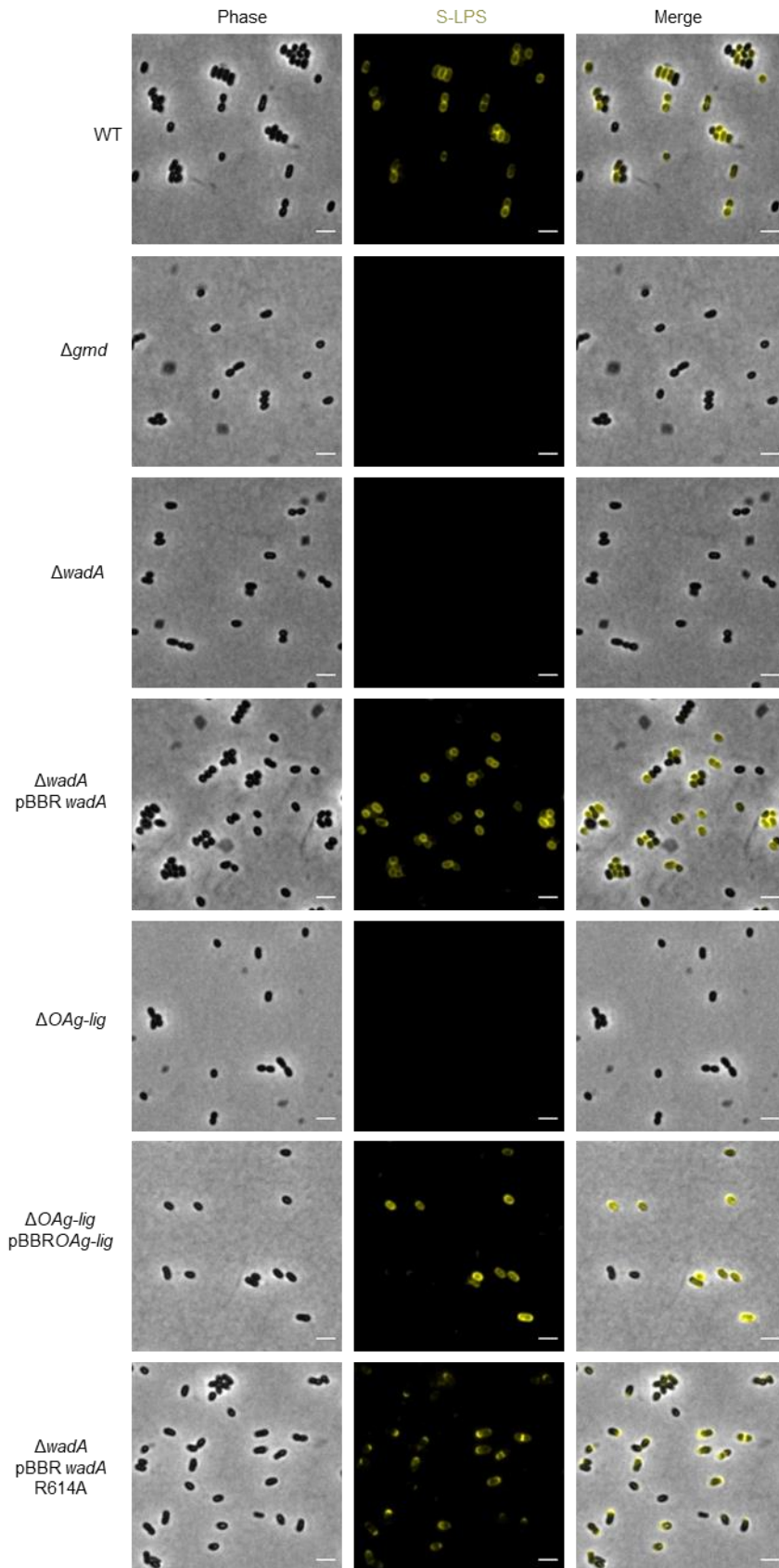


Figure S7 | S-LPS labelling of *B. melitensis* 16M strains. *B. melitensis* strains were labelled with primary mAb targeting the O-antigen (A76/12G12) and a goat anti-mouse antibody coupled to Alexa fluor 514 to detect S-LPS. Δgmd was used as a negative control. The experiment was performed in three biological replicates (n=3). Scale bar is 2 μ m. Source data are provided as a Source Data file.

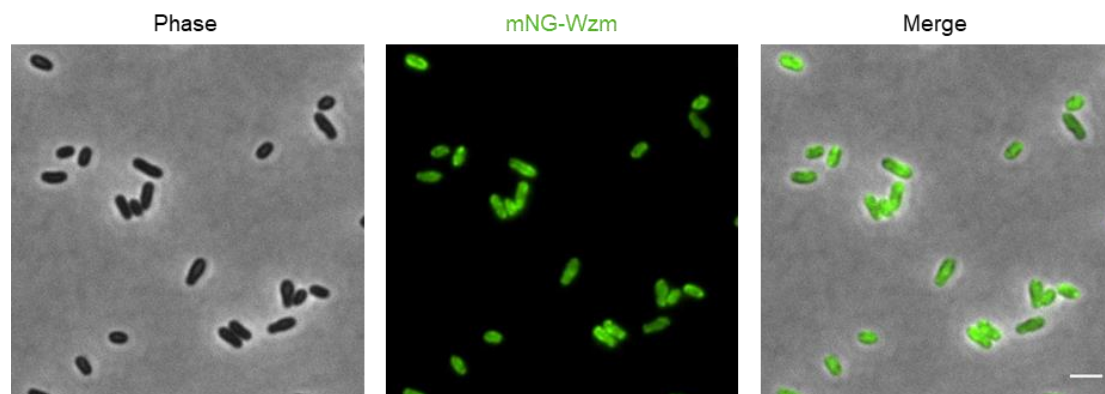


Figure S8 | Wzm appears dispersed surrounding the bacterial cell. Representative image for *mNG-wzm* observed in exponential phase of growth for one biological replicate (n=3). Scale bar is 2 μ m. Source data are provided as a Source Data file.

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