Supplementary Information for
Identification of the *Bartonella* autotransporter CFA as protective antigen and hypervariable target of neutralizing antibodies in mice.

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- Supplementary Material & Methods
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Bacterial strains and growth conditions. All bacterial strains and plasmids used in this study can be found in the supplementary table S1 and S2. 

_E. coli_ strains were cultivated in lysogeny broth (LB) or solid agar plates supplemented with the appropriate antibiotics at 37°C overnight. 

_Bartonella_ strains were grown at 35°C and 5% CO2 on Columbia blood agar (CBA) plates supplemented with 5% defibrinated sheep blood (Oxoid Cat#SR0051) and the appropriate antibiotics. _Bartonella_ stocks are maintained at -80°C and are streaked as “thumbnail” for 3 days and are subsequently expanded for 2 days prior to the experiment. 

Plasmids were introduced into _Bartonella_ strains by conjugation from _E. coli_ strain β2150 using biparental mating (1). 

Antibiotics or supplements were used in the following concentrations: ampicillin (AppliChem, Cat# A0839) and kanamycin (AppliChem, Cat#A1493) at 30 μg/ml, gentamicin (AppliChem, Cat#A11492) at 10 μg/ml, streptomycin (AppliChem, Cat#A1852) at 100 μg/ml, diaminopimelic acid (DAP, Merck, Cat#D1377) at 1 mM.

Constructions of strains and plasmids. DNA manipulations were performed according to standard techniques and all cloned inserts were DNA sequenced to confirm sequence integrity. For the generation of bacterial knock outs the previously described two-step selection procedure for gene replacement was used (2). In brief, a suicide vector containing both a kanamycin cassette for positive selection and the rpsL gene was mobilized into the desired gene locus of _B. taylorii_ IBS296 SmR. In subsequent negative selection using streptomycin, bacteria which had undergone a second homologous recombination event and thus had lost the cointegrated mutagenesis plasmid were selected. The resulting colonies carried either the WT (revertant) or the desired deletion allele. Colony PCR was performed to identify and differentiate the two genotypes. For complementation overexpression selected genes from plasmid were cloned into variants of the plasmids pJS43 (3) under the control of the AphT promoter of pJC43 (GFP-expression) or IPTG-inducible promoter Plac(MQ5) (1) (CFABtay). For genetic complementation under the natural promoter in the chromosome insertions via the Tn7 transposon were used (based on pUC18T-mini-Tn7, (4)). For antibody expression vectors, the corresponding V regions of heavy and light chain were cloned into the CMV-promoter-driven mammalian expression vector pXLG1.2, followed by the Cy2a constant domain (kindly provided by Prof. Shozo Izui, University of Geneva). A detailed description for the construction of each plasmid is presented in Table S1 and S2. The sequences of all oligonucleotide primers and synthesized gene fragments used in this study are listed in Table S3 and S4.

Mouse strains and husbandry. Female BALB/cJRj and C57BL/6JRj mice were purchased from Javier Labs. 

The genetically modified strains AID/- (5), C3/- (6), JHT/- (7), KbDb/- (8), MHCII/- (9), Rag1/- (10), slgM/- (11), TCRβδ/- (12) and T11μMT (13) were bread at the Laboratory Animal Science Center (LASC, University of Zurich, Switzerland) under SPF conditions. FcyR4alpha x C3 -/ (crossing of C3/- with FcyR4alpha/- (14)) were bread at the Transgenic Mouse Core Facility (TMCF, University of Basel, Switzerland). μMT/- mice (15) were obtained from Jackson Laboratories, Maine, USA. The strains CD1d/- (16) and MR1/- (17) were a kind gift from Prof. Genaro De Libero, University of Basel. AID x C3/- was obtained by crossing AID/- and C3/- . TCRβ/- and TCRδ/- single knock outs were obtained by back-crossing TCRβδ/- with WT C57/BL6. CD1d/- x MHCII/- and MR1/- x MHCII/- were obtained by crossing the respective line with MHCII/- mice.

All mice were in C57BL/6 background unless specified otherwise. Adult mice (5-8 weeks) of both sexes were used for experiments.

Animal experimentation. Animals were infected or immunized i.d. with 10^7 cfu bacteria in PBS under isofluorane/oxygen anaesthesia. In long-term experiments with repeated blood sampling a
proportionally small number of animals were lost from follow-up prior to the final time point and were excluded from the analysis. Blood was drawn in 3.8% sodium citrate at the indicated days post infection. For blood cfu count, whole blood was frozen at -80°C, thawed and plated in limited dilution series on CBA blood agar. For serum analysis, the blood samples were centrifuged for 5 min at RT at 5000 x g in serum tubes. Serum was frozen at -20°C until further usage. To obtain a pool of immune serum for in vitro analysis and adoptive transfer to mice, blood samples were collected from 10 randomly selected C57BL/6 mice on day 45 after confirmed bacteremic infection and the serum was pooled for further use. Naïve or immune sera were injected i.v. in 100 µl. Antibodies were given i.v.in a dose of 250 µg in PBS.

Erythrocyte infection and EAI assay. Murine Erythrocytes were isolated from BALB/c mice after blood collection in 3.8% sodium citrate by terminal bleeding. Erythrocytes were purified using a Ficoll-gradient and kept at 4°C in DMEM (Gibco, Cat#61965) supplemented with 10% FCS (Amimed, Cat#2-01F30I) for up to two weeks until usage. For the EAI assay, a serial dilution of sera or antibodies was performed in a U-bottom 96-well plate in DMEM with 10% FCS. 5*10^5 bacteria (GFP+) were added per well and incubated for 1 h at 35°C and 5% CO2. 10^5 (MOI 0.5) erythrocytes were added in 100 µl DMEM containing 10% FCS. After 24 h the supernatant was removed and the cells were fixed in 1% PFA and 0.2% Gluturaldehyde in PBS for 10 min at 4°C. FACS buffer (2% FCS in PBS) was added and the plates were analyzed by flow cytometry (BD Cantoll, using the HTS autosampler). If indicated, the serum was heat-inactivated at 60°C for 30 min before usage. EAI titers were calculated by endpoint titer determination as described (18).

For confocal microscopy, erythrocytes were infected using the same conditions lacking the serum pre-incubation.

Microscopy. Bacteria were grown as described above, collected in PBS and stained with purified antibody for 45 min at RT. Bacteria were then washed twice with PBS, centrifuged at 4000 x g for 5 min and stained with the secondary antibody (Anti-mouse IgG ALEXA 488, Biolegend, Cat#405319; Poly4053) for 1 h at 4°C in the dark. After 3 washes, the bacteria were stained with DAPI (Invitrogen, Cat# D1306) for 1 h at 4°C in the dark and after another three washes fixed in 3.7% PFA for 10 min at 4°C in the dark. Erythrocytes were infected with GFP+ bacteria as described above. Erythrocytes were then stained with anti-Ter119-Alexa 647 (Biolegend, Cat#116218) for 1 h at 4°C in the dark. After washing and centrifugation at 100 x g, 5 min, the cells were fixed in 1% PFA and 0.2% Gluturaldehyde in PBS for 10 min at 4°C in the dark. Fixed erythrocyte samples were centrifuged at 100 x g for 5 min. Stained bacteria samples were centrifuged at 4000 x g for 5 min. For both types of samples, the supernatant was removed and the cell pellet was resuspended in ProLong Diamond Antifade mountant (Invitrogen, Cat#P36961. 15 µl of the suspension was applied on 18 mm, #1.5 thickness coverslips and mounted onto glass slides. After 24 h of curing at room temperature, the coverslips were sealed with nail polish. Confocal images were acquired with a SP8 confocal microscope (Leica) equipped with 488 and 638 nm solid-state lasers and a Plan Apo CS2 63x, 1.40 NA oil objective. 3D-SIM was performed using a DeltaVision OMX-Blaze system (version 4; GE Healthcare) equipped with 488 and 568 nm solid-state lasers, Plan Apo N 63x, 1.42 NA oil objective and 4 liquid-cooled sCMOs cameras (pco Edge, full frame 2560 x 2160; Photometrics). Optical z-sections were separated by 0.125 µm. Exposure times were between 3 and 10 ms, with three rotations of the illumination grid. Multichannel imaging was achieved through sequential acquisition of wavelengths by separate cameras. First, the channels were aligned in the image plane and around the optical axis using predetermined shifts and measured using a target lens and the SoftWoRx alignment tool. Afterwards, they were carefully aligned using alignment parameters from control measurements made with 0.5 µm diameter multi-spectral fluorescent beads. Raw 3D-SIM images were processed and reconstructed using the DeltaVision OMX SoftWoRx software package. The final voxel size was 40 nm x 40 nm x 125 nm.

Gentamicin protection assay. Blood from infected animals (day 14 post infection) or over night in-vitro infected erythrocytes (see above) were incubated for 2 h at 35°C, 5% CO2 either in PBS
alone or in PBS containing 40 µg/ml gentamicin. Cells were washed 3 times with PBS and centrifugation at 300 x g for 5 min. Cell pellets were frozen at -80°C for erythrocyte lysis and plated in serial dilutions on blood agar plates. Supernatant and medium only controls were directly used for serial dilution and plating.

**Antibody binding to the bacterial surface by flow cytometry.** Bacteria were grown as described above, collected in PBS and stained with purified antibody for 45 min at RT. Bacteria were then washed twice with PBS, centrifuged at 4000 x g for 5 min and stained with the secondary antibody (Anti-mouse IgG-Alexa 647, Biolegend, Cat#405322; Poly4053) for 1 h at 4°C in the dark. After 3 washes, the bacteria were fixed in 3.7% PFA for 10 min at 4°C in the dark and resuspended in FACS buffer before analysis via flow cytometry (BD CantoII). For CFA expression from plasmid, the bacteria were incubated in DMEM containing 10% FCS and 100 nM IPTG overnight prior to staining.

For binding curves, a serial dilution series of monoclonal antibody or isotype was performed in a U-bottom 96-well plate. 10^7 bacteria were added per well. After 45 min at RT in the dark, staining continued as described above.

**ELISA.** Purification of *Bartonella* outer membrane proteins (OMP) was performed as has been described previously (19). For antibody titers in serum, anti murine IgG-HRP or anti murine IgM-biotin (Anti-mouse IgG-HRP, Biolegend, Cat#405306; Poly4053; Anti-mouse IgM biotin, Biolegend, Cat#46503; RMM-1) and streptavidin-HRP (Biolegend, Cat#405210) were used. In brief, ELISA plates were coated over night with purified *Bartonella* OMP (1 mg per 96-well plate) in coating buffer (Biolegend). The plates were washed with 0.05% Tween in PBS and blocked 1 h at RT with 1x ELISA buffer (Biolegend). After another wash, sera were added in dilution series for 3 h at RT. After 3 washes, detection antibody was added in the recommended dilution for 1 h at RT. If necessary streptavidin-HRP was added after another 3 washes for 30 min at RT. After 5 washes 1x ELISA substrate was added at RT for 5-10 min and stopped with 1 M phosphoric acid. Plates were read at 450 and 570 nm using a plate reader.

**Hybridoma production and handling.** For hybridoma production the DiSH Kit (Enzo Life Sciences, Cat# ENZ-71001-0001) was used. BALB/c mice were infected as described above before i.v. reinfection with 10^7 cfu after clearance of the first infection. 2 weeks later, the animals were boosted with 10^7 cfu heat inactivated bacteria (3 h at 60°C) and the spleens were harvested 2 days later. A collagenase digest (3 mg/ml Collagenase IV, 2% FCS, in RPMI, Givco Cat# 21875034) was performed for 30-60 min at 37°C. The digestion mixture was put through a cell strainer and approx. 10 ml of RPMI + 10% FCS were added. The cells were centrifuged for 10 min at 300 x g at 4°C and a red blood cell lysis was performed by resuspending the pellet in ACK buffer (150 mM NH4Cl, 10 mM KHCO3 and 0.1 mM EDTA). After 1 min at RT, 10 ml of RPMI + 10% FCS were added and the cells were centrifuged again and used according to the DiSH Kit protocol. In brief, the obtained lymphocytes were washed with WCM. Myeloma fusion partner SP2ab (Enzo Life Sciences, Cat#ENZ-70008-0001) grown in PMC medium, washed once with WCM and added to the lymphocyte pellet in a ration of 1:5 Sp2ab : lymphocyte. After centrifugation for 10 min at 4°C, 300 x g, the cell pellet was warmed to 37°C in a water bath. While keeping the pellet in the water bath, PEG1000 was added drop wise to the cell mixture. After 60 sec at 37°C, 4 ml of prewarmed WCM were added drop-wise over the course of 2 min while keeping the cells at 37°C. Another 5 ml were added over the course of 1 min. Another 6 ml were added over the course of 1 min. After 10 min at 37°C, 30 ml of FCM were added and the cells were centrifuged at 150 x g for 5 min. The cells were then incubated over night in FCM at 37°C, 5% CO2. The fused cells were plated in semisolid medium complemented with hybridoma selection medium until the appearance of single colonies (AbeoClone™ (HAT Semi-solid Medium), Enzo Life Sciences, Cat# ENZ-70004-0090), approx. after 8 days. Clones were then harvested in RCM, expanded in ECM and screened for specificity using the EAI assay. Hybridoma clones were then grown in RPMI complemented with 10% FCS.
**Purification of monoclonal antibodies.** Sequencing of the antibody expressed by a hybridoma cell line was performed by Absolute Antibody, UK. The sequences of LS4G2 and LS5G11 V\_\_ and V\_\_\_ region are given in Table S4.

The corresponding gene fragments were synthesized (Genscript, New Jersey, USA). For recombinant expression, rearranged V regions were subcloned into the CMV-promoter-driven mammalian expression vector pXLG1.2, followed by the Cy2a constant domain (kindly provided by Prof. Shozo Izui, University of Geneva), corresponding to the Genbank sequences for mouse IgG2a (J00470.1). The procedure for the light chain was identical.

Antibodies were expressed by transient co-transfection of HEK cells (Protein Production and Structure Core Facility, EPFL, Lausanne, Switzerland) and purified on protein G columns (GE healthcare, Cat# GE29-0485-81) with AKTAprime plus followed by PBS dialysis.

For conjugation of the purified antibody, we used the lightning link labeling kit from Expedeon according to the manufacturer’s protocol.

**Immunoprecipitation.** *Bartonella taylorii* IBS296 was grown as described above. Bacteria were collected in wash buffer (50 mM HEPES pH 7.4, 200 mM NaCl, 5% glycerol) containing benzoase (Merck, Cat#E1014) and protease inhibitors (cOmplete™ Mini EDTA-free protease inhibitor cocktail, Roche, Cat#11836170001), lyzed using French Press and centrifuged at 10,000 x g for 10 min at 4°C. The supernatant was centrifuged at 100,000 x g at 4°C to obtain the membrane protein fraction. The pellet was solubilized overnight at 4°C with 1% DDM (n-dodecyl-\-D-maltoside) in wash buffer and again centrifuged at 100,000 x g at 4°C. The supernatant was incubated overnight with 20 µg of antibody and 80 µL of UltraLink Resin (Thermo Scientific, Cat# 53132) at 4°C. The mixture was centrifuged at 6000 x g and the beads were washed 3 times with excess wash buffer containing 0.05% DDM before elution with 25 µL of Laemmli buffer (BioRad). If indicated the samples were digested using 1 U of proteinase K (Macherey-Nagel, Cat# 300297) for 10 min at 4°C before another 3 washing steps and elution.

**Sample preparation for MS-based proteome analysis.** 0.5 µL of 1 M iodoacetamide (Sigma-Aldrich, Cat#I1149) was added to the samples. Cysteine residues were alkylated for 30 min at 25°C in the dark. Digestion and peptide purification was performed using S-trapTM technology (Protifi) according to the manufacturer’s instructions. In brief, samples were acidified by addition of 2.5 µL of 12% phosphoric acid (1:10) and then 165 µL of S-trap buffer (90% methanol, 100 mM TEAB (Triethylammonium bicarbonate) pH 7.1) was added to the samples (6:1). Samples were briefly vortexed and loaded onto S-trapTM micro spin-columns and centrifuged for 1 min at 4000 g. Flow-through was discarded and spin-columns were then washed 3 times with 150 µL of S-trap buffer (each time samples were centrifuged for 1 min at 4000 g and flow-through was removed). S-trap columns were then moved to the clean tubes and 20 µL of digestion buffer (50 mM TEAB pH 8.0) and trypsin (at 1:25 enzyme to protein ratio) were added to the samples. Digestion was allowed to proceed for 1 h at 47°C. After, 40 µL of digestion buffer was added to the samples and the peptides were collected by centrifugation at 4’000 g for 1 minute. To increase the recovery, S-trap columns were washed with 40 µL of 0.2% formic acid in water (400g, 1 min) and 35 µL of 0.2% formic acid in 50% acetonitrile. Eluted peptides were dried under vacuum and stored at -20 °C until further analysis.

The peptides were dissolved in LC-buffer A (0.15% formic acid, 2% acetonitrile) right before ultrasonication for 10 sec and shaking at 1’400 rpm at 25°C for 5 min.

**Mass spectrometry.** For each sample, aliquots of 0.4 µg of total peptides were subjected to LC-MS analysis using a dual pressure LTQ-Orbitrap Elite mass spectrometer connected to an electrospray ion source (both Thermo Fisher Scientific) and a custom-made column heater set to 60°C. Peptide separation was carried out using an EASY nLC-1000 system (Thermo Fisher Scientific) equipped with a RP-HPLC column (75 µm x 30 cm) packed in-house with C18 resin (C18 resin (ReproSil-Pur C18–AQ, 1.9 µm resin, Dr. Maisch GmbH, ReproSil-Pur C18–AQ) using a linear gradient from 95% solvent A (0.1% formic acid in water) and 5% solvent B (80% acetonitrile, 0.1% formic acid, in water) to 35% solvent B over 50 minutes to 50% solvent B over 10 minutes to 95% solvent B over 2 minutes and 95% solvent B over 18 minutes at a flow rate of 0.2 µl/min. The data acquisition mode was set to obtain one high resolution MS scan in the FT
part of the mass spectrometer at a resolution of 120'000 full width at half maximum (at 400 m/z, MS1) followed by MS/MS (MS2) scans in the linear ion trap of the 20 most intense MS signals. The charged state screening modus was enabled to exclude unassigned and singly charged ions and the dynamic exclusion duration was set to 30 s. The collision energy was set to 35%, and one microscan was acquired for each spectrum. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (20) partner repository with the dataset identifier PXD028783 and 10.6019/PXD028783.

Protein Identification and Label-free Quantification. The acquired raw-files were imported into the Progenesis QI software (v2.0, Nonlinear Dynamics Limited), which was used to extract peptide precursor ion intensities across all samples applying the default parameters. The generated mgf files were searched using MASCOT against a decoy database containing normal and reverse sequences of B. taylorii IBS296 (UniProt, 10.01.2020) proteome and commonly observed contaminants (in total 3484 sequences) generated using the SequenceReverser tool from the MaxQuant software (Version 1.0.13.13). The following search criteria were used: full tryptic specificity was required (cleavage after lysine or arginine residues, unless followed by proline); 2 missed cleavages were allowed; carbamidomethylation (C) was set as fixed modification; oxidation (M) and protein N-terminal acetylation were applied as variable modifications; mass tolerance of 10 ppm (precursor) and 0.6 Da (fragments) was set. The database search results were filtered using the ion score to set the false discovery rate (FDR) to 1% on the peptide and protein level, respectively, based on the number of reverse protein sequence hits in the datasets. Quantitative analysis results from label-free quantification were normalized and statistically analyzed using the SafeQuant R package v.2.3.4 to obtain protein relative abundances. This analysis included summation of peak areas per protein and LC MS/MS run followed by calculation of protein abundance ratios. Only isoform specific peptide ion signals were considered for quantification. The summarized protein expression values were used for statistical testing of differentially abundant proteins between conditions. Here, empirical Bayes moderated t-Tests were applied, as implemented in the R/Bioconductor limma package. The resulting p-values were adjusted for multiple testing using the Benjamini Hochberg method.

DNA extraction and sequencing. Genomic bacterial DNA was extracted with QIAGEN Genomic-tip 20/G according to the manufacturer’s guidelines. Whole genome sequencing of B. taylorii M1 strain was done with Illumina and Nanopore sequencing technologies. Illumina short-read sequencing was performed at the Microbial Genome Sequencing Center (MiGS) using the Illumina NextSeq 2000 platform. The libraries for the Nanopore sequencing were made with Ligation Sequencing Kit (Oxford Nanopore Technologies, Cat#SQK-LSK109) and Native Barcoding Expansion 1-12 (Oxford Nanopore Technologies, Cat#EXP-NBD104) according to the protocol provided by ONT. The sequencing was done with the MiniION Mk1B sequencing device and the MiniION Flow Cell (R10.3).

Genome assembly and annotation. All calculations were performed at sciCORE scientific computing center at the University of Basel. Base-calling and barcoding of raw ONT data was done with Guppy v.5.0.7 (Oxford Nanopore Technologies). Then, ONT read sets were filtered and reads shorter than 1 kbp were removed with Filtlong v.0.2.0 (21). Illumina reads were quality assessed with FastQC v.0.11.8 (22) and if needed trimmed with Trimomatic v.0.39. Next, consensus assemblies were generated with Trycycler v.0.5.0 (23) based on multiple input assemblies made with Canu v.2.1.1 (24), Raven v.1.5.0 (25), Minimap2 v.2.20-r1061 (26) combined with Miniasm v.0.3-r179 (27) and polished with Minipolish v.0.1.3 (28). The assemblies then were finalised by polishing with ONT reads (Medaka v.1.4.3) and short reads (Pilon v.1.24, (29)). The genomes for B. taylorii IBS296 and M1 can be found under the following accession numbers respectively: CP083444 and CP083693.

Bioinformatic analysis. Synteny analysis of Cfa autotransporter was inferred with MultiGeneBlast v.1.1.14 (30) in architecture search mode with default search parameters. The
database was generated in MultiGeneBlast from annotated genome sequences of *Bartonella* species (Table S5).

Search for domains present in CFA was done with Conserved Domain Search tool (31) with Result mode set on "Full".

The alignment of protein sequences of Cfa was made with MSAProbs v.0.9.7 (32) implemented in MPI Bioinformatics Toolkit. Positions of the alignment containing at least 40% of gaps were removed from the alignment with Mask alignment option of Geneious Prime. Resulting trimmed alignment was used to calculate conservation scores with the Property Entropy (PE) scoring method of Protein Residue Conservation Prediction tool (33). Conservation scores were summed and averaged within a sliding window of 19 amino acids [ai-9; ai+9], where a is a conservation score and i is an amino acid index. The resulting values were plotted with GraphPad Prism.

Alignment of Cfa from lineage 4 species was used to identify variability sites. Sites were considered variable if averaged PE scores were equal or lower 0.4. Then, variability sites were mapped onto Cfa from *B. taylorii* IBS296 and alignments generated for *B. quintana*. Modelling the Cfa from *B. taylorii* IBS296 was done with AlphaFold (34) with default options.

Tree topology and bootstrap support values were inferred with PhyML v.3.0, model (JTT+G+I+F) (35), 1000 bootstrap replicates. The tree was based on a concatenated alignment of five core protein sequences (FtsZ, GroEL, GyrB, RpoB and SecY). The protein sequences were aligned with Clustal Omega (implemented in Geneious Prime), then concatenated, and all gaps were removed with Mask alignment option of Geneious Prime.

**Data analysis.** Unless stated differently, statistical analysis of the obtained data was performed using GraphPad Prism Software and the statistical tests as indicated. P-values are depicted as follows: ns = P > 0.05; * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

All LC-MS analysis runs were acquired from independent biological samples. To meet additional assumptions (normality and homoscedasticity) underlying the use of linear regression models and Student t-Test MS-intensity signals are transformed from the linear to the log-scale.

Unless stated otherwise linear regression was performed using the ordinary least square (OLS) method as implemented in base package of R v.3.1.2. The sample size of three biological replicates was chosen assuming a within-group MS-signal Coefficient of Variation of 10%. When applying a two-sample, two-sided Student t-test this gives adequate power (80%) to detect protein abundance fold changes higher than 1.65, per statistical test. Note that the statistical package used to assess protein abundance changes, SafeQuant, employs a moderated t-Test, which has been shown to provide higher power than the Student t-test. We did not do any simulations to assess power, upon correction for multiple testing (Benjamini-Hochberg correction), as a function of different effect sizes and assumed proportions of differentially abundant proteins. For peptide enrichment upon proteinase K digest, the ratio between digested and undigested sample was formed and plotted within a sliding window of 19 amino acids [ai-9; ai+9], where a is a conservation score and i is an amino acid index.
Fig. S1. Complement-independent EAI activity and lack of bactericidal effect of antisera and gentamicin-insensitivity of intraerythrocytic Bartonella. (A) Flow cytometry analysis of wild-type (WT) and GFP-expressing *B. taylorii* IBS296. (B) EAI assay comparing untreated immune serum and heat-inactivated (60°C, 30 min) immune serum. (C) Bacterial survival in the EAI assay. Bacteria were plated at the end of an EAI assay comparing the adhesion-inhibiting activity of naïve serum, anti-*B. taylorii* IBS296-immune serum and heat-inactivated anti-*B. taylorii* IBS296-immune serum. (D) Decay of EAI titer in the serum of B-cell knock-out mice receiving immune serum transfer in the experiment reported in Figure 1C, D (100 µl immune serum into infected), side-by-side with titers determined in a separate group of uninfected B-cell knock-out mice that received the same serum transfer (100 µl immune serum into naïve) and a group of uninfected B-cell knock-out mice without serum transfer (no treatment). The vertical dotted line indicates the time point of serum transfer. A baseline sample was collected prior to serum transfer on d87. (E) Lysis control for the gentamicin (Gm) protection assay shown in Figure 2E. Blood was collected from infected wild-type (WT) mice on day 14 p.i.. Cfu/ml counts are shown for untreated blood samples, Gm-treated erythrocytes from infected animals and erythrocytes which had been lysed by a freeze-thaw cycle prior to Gm treatment. All data show representative results from two independent experiments; in vitro experiments were performed in technical triplicates and in vivo experiments with at least 3 mice per group and experiment, (B) shows the mean ± SD of technical triplicates. Data are displayed as mean ± SD. Statistical analysis was performed using one-way ANOVA, ns: P > 0.05; HI = heat inactivated. Related to figures 1 and 2.
Fig. S2. Antibody response and *B. taylorii* IBS296 bacteremia in mice lacking AID, soluble IgM, C3 and CD40L or combinations thereof. (A) IgM (right Y-axis) and IgG (left Y-axis) antibodies binding to the *B. taylorii* IBS296 (Bta) outer membrane proteins (OMPs) fraction were measured by ELISA. Sera were collected from wild-type (WT), AID−/−, *slgM−/−*, AID−/−*x slgM−/−* and B-cell knock-out mice on day 42 days after infection. (B) We infected AID−/−* x C3−/−* mice with *B. taylorii* IBS296 and measured bacteremia and serum EAI titer over time. (C-E) Bacteremia and EAI serum titers of individual CD40L−/− mice. Each plots represents one single animal from the experimental group reported in Figure 3G. (A-B) Representative data from two independent experiments with (A) and (B) showing the mean±SD of at least three animals per group. (A, B) Data are represented as mean ± SD; n.d. = not detected. Related to figure 3.
Fig. S3. Characterization of monoclonal antibodies targeting CFA and conservation of this autotransporter within the Bartonellae. (A) Survival of *B. taylorii* IBS296 in the EAI assay. Bacteria were incubated with either one of the monoclonal antibodies LS4G2 and LS5G11 or with medium control. Surviving bacteria were quantitated 24 h later. (B) Staining of *B. taylorii* IBS296, *B. quintana*, *B. tribocorum*, *B. taylorii* M1, *B. grahamii* and *B. birtlesii* with LS4G2 (red), LS5G11 (blue) or irrelevant isotype control antibody (grey) was compared by flow cytometry. Unstained bacteria of each species (black) served as background control. (C, D) Flow cytometry staining of isogenic strains of (C) *B. taylorii* M1 or (D) *B. tribocorum*, either in their unmodified wild-type (WT) form or transformed with either empty vector control (*pempty*) or plasmid *pcfaBtay* driving expression of CFA from *B. taylorii* IBS296. Bars show the percentage of bacteria stained with LS4G2 or LS5G11, respectively, as determined by flow cytometry. All data show representative results from three independent experiments, which were performed in technical triplicates. Data are represented as mean ± SD. Data presented in (A) were analysed 1-way ANOVA. *ns* = *P* > 0.05. (E) Phylogeny of the *Bartonella* genus and the related *Brucella melitensis* and "*Candidatus Tokpelaia hoelldobleri*" as outgroup taxons. Filled and empty circles show the presence or absence, respectively, of genes encoding the CFA autotransporter. Colouring scheme represents lineages of *Bartonella*, orange (L1), red (L2), green (L3) and yellow (L4). Values above nodes show bootstrap support (>75%). The scale bar indicates the number of substitutions per site. Related to figures 4 and 5.
Fig. S4. Sequence variation in CFA between and within individual *Bartonella* lineages and species. Protein sequence alignments for (A) *B. taylorii* IBS296 and M1, another *B. taylorii* isolate, (B) representative species of *Bartonella* lineage 4 and (C) a collection of clinical isolates of *B. quintana*. Black indicates high sequence conservation, β-helices (stalk) are labelled in blue and in the β-barrel in green. Hyper-variable regions are highlighted in red and the linker between β-helices is additionally highlighted in orange.
Fig. S5. Structure prediction for the N-terminal stalk domain of CFA. (A) Schematic overview of the CFA domain topology. The N-terminal head domain (orange) is followed by two β-helices (blue) connected by an unstructured linker. A C-terminal β-barrel (green) anchors the structure to the outer membrane. Red lines indicate variability regions in the N-terminal β-helix as determined by sequence alignment. AC, autochaperone domains. (B) A three-dimensional structure prediction of CFA’s β-helix 1 from *B. taylorii* IBS296 was generated using AlphaFold2. The model indicates a conserved pertactin-like fold with a rigid core formed by several turns of β-strands (blue). The modeling approach indicated a number of regions that likely fall out of register of the β-helix, which suggests that they protrude to the outside and decorate the stalk (R1-R10, dotted orange lines). (C) Schematic representation of the AlphaFold2 model shown in (B). 51 β-strands forming the core of the helix are shown as blue arrows. R1-R10 regions are indicated as orange loops. The locations of the hypervariability regions (V1-V8) are shown in red. The location of the putative LS4G2 binding site is depicted in green. Amino acid residues comprised in the respective regions are indicated. Related to figure 6.
**Table S1.** Bacterial strains used for this study.

| Strain description                                                                 | Source                        | Strain number |
|-----------------------------------------------------------------------------------|-------------------------------|---------------|
| Wild-type (Sm<sup>+</sup> strain) of *B. taylorii* IBS296 (serving as WT)          | (36)                          | LSB001        |
| *B. taylorii* GFP+ (harbouring pLS04)                                              | This paper                    | LSB115        |
| *B. taylorii* Δcfa                                                                 | This paper                    | LSB311        |
| *B. taylorii* revertant                                                            | This paper                    | LSB310        |
| *B. taylorii* Δcfa; pca, harbouring pLS51                                           | This paper                    | LSB320        |
| *B. taylorii* Δcfa; cfa::Tn7                                                       | This paper                    | LSB424        |
| Wild-type *B. birtlesii* IBS325                                                    | (37)                          | LSB002        |
| Wild-type *B. quintana* JK31                                                       | (38)                          | LSB045        |
| Wild-type *B. taylorii* M1                                                         | This paper; kind gift from    | LSB208        |
| *B. taylorii* M1 pcfa; harbouring pLS51                                             | Richard Birtles               |               |
| *B. taylorii* M1 pempty; harbouring pLS60                                            | This paper                    | LSB317        |
| Wild-type (Sm<sup>+</sup>) strain of *B. tribocorum*, IBS509 (serving as WT)       | (2)                           | LSB009        |
| *B. tribocorum* pcfa; harbouring pLS51                                              | This paper                    | LSB274        |
| *B. tribocorum* pempty; harbouring pLS60                                             | This paper                    | LSB314        |
| *B. tribocorum* cfa::Tn7                                                           | This paper                    | LSB401        |
Table S2. List of plasmids used in this study

| Plasmid      | Purpose                        | Resistance | Cloning strategy | Generation | Source                                                                 |
|--------------|--------------------------------|------------|------------------|------------|------------------------------------------------------------------------|
| pBZ485       | Generation of pLS51            | Kanamycin  | -                | -          | (1)                                                                    |
| pXLG1.2      | Generation of pLS06, 09, 34 and 35 | Ampicillin | -                | -          | (39), kindly provided by Prof. Shozo Izui, University of Geneva       |
| pJC43        | Generation of pLS04            | Kanamycin  | -                | -          | (3)                                                                    |
| pPG1000      | Generation of pLS04            | Gentamycin | -                | -          | (40)                                                                   |
| pTR1000      | Generation of pLS25            | Kanamycin  | -                | -          | (2)                                                                    |
| pUC18T-mini-Tn7 | Generation of pJS208        | Gentamycin | -                | -          | (4)                                                                    |
| pLS04        | Constant GFP expression in B. taylorii | Gentamycin | In-fusion ligation | Exchange of the kanamycin resistance cassette in pJC43 with a gentamycin cassette (pPG1000) | This study |
| pLS06        | LS4G2 IgG2a heavy chain expression | Ampicillin  | Restriction digest using NotI and MfeI | Exchange of previous V-region of pXLG1.2 with synthesized gene fragment LS4G2 VH | This study |
| pLS09        | LS4G2 kappa light chain expression | Ampicillin  | Restriction digest using NotI and HindIII | Exchange previous V region of pXLG1.2 with synthesized gene fragment LS4G2 VL | This study |
| pLS25        | Generation of B. taylorii Δcfa | Kanamycin  | Restriction digest using SalI and BamHI | Insertion of homology regions 1 and 2 into pTR000 | This study |
| pLS34        | Expression of LS5G11 IgG2a heavy chain | Ampicillin  | Restriction digest using NotI and MfeI | Exchange of previous V-region of pXLG1.2 with synthesized gene fragment LS5G11 VH | This study |
| Plasmid | Description | Selection | Transformation | Notes |
|---------|-------------|-----------|----------------|-------|
| pLS35  | LS5G11 kappa light chain expression | Ampicillin | Restriction digest using NotI and HindIII | Exchange previous V region of pXLG1.2 with synthesized gene fragment LS5G11 Vκ |
| pLS51  | IPTG inducible expression of *cfa* in *Bartonella* | Kanamycin | In-fusion ligation | Ligation of JC43 backbone with P*lac* of pBZ485 and *cfa* gene |
| pLS60  | Empty vector control for pLS51 | Kanamycin | In-fusion ligation | Amplification of pLS51 lacking *cfa* insert |
| pLS80  | Insertion of *cfa* containing its natural promoter into *Bartonella* using Tn7 transposon | Kanamycin, ampicillin | In-fusion ligation | *cfa* including its putative natural promoter was ligated into Xmal and HindII digested pJS208 |
| pJS208 | Intermediate for pLS80 | Kanamycin, ampicillin | In-fusion ligation | KanR gene and *PaphT* of JC43 and *tetR*-P*tetR* were ligated into EcoRV and KpnI digested pUC18T-mini-Tn7 |
**Table S3.** List of oligonucleotides used in this study.

| Fw primer | Rev primer | Amplified target | Purpose |
|-----------|------------|-------------------|---------|
| prJS370: 5'CTCATCCTGTCTTTGATCAG ATC | prJS371: 5'AGATCTGGGGTTCGAA ATGACCG | pJC43 excluding KanR | Generation of pLS04 |
| prJS372: 5'TCAAGAGACAGGATGAAAG CCGTCGCCAAGTAAACTGG | prJS373: 5'TTTCGAACCCCAGATGACCG | Gentamycin cassette of pPG1000 | Generation or pLS04 |
| prLS213: 5'CATCTCTAGCAGCAGCCGCTTGTCT | prLS214: 5'CTCAAAACTTTCTAAATA TAATTGCTAAAGG | Homology region 1 cfa locus of LSB001 | Generation or pLS25 |
| prLS230: 5'ATGCAAATTATATTGAGAAG TTTGACATCTGCATGATAGCT ATGTTGG | prLS231: 5'TGCAAGCTTAGCTCT GCAAGCTGACAAATAA CCAACCTCTCGTCTCCG | Homology region 2 cfa locus of LSB001 | Generation or pLS25 |
| prLS213: 5'CATCTCTAGCAGCAGCCGCTTGTCT | prLS231: 5'TGCAAGCTTAGCTCT GCAAGCTGACAAATAA CCAACCTCTCGTCTCCG | SOEing PCR homology region 1 and 2 cfa locus | Generation or pLS25 |
| prJS1003: 5'GGCTTATAGACCCACTCA CTGCCCGCTTCTCAG | prJS1004: 5'CATGATGACATTGTC ATGTTGG | Amplification of regulatory region including lacI and P\_lac of pBZ485 | Generation of pLS51 |
| prJS1005: 5'GAGGGAACATATGAATTATGT ATTAAAAAGCGTGACACG | prJS1006: 5'CTCTCAATGCGCGACTGACTAAGG ATTTCCG | Amplification of cfa or LSB001 | Generation of pLS51 |
| prJS1007: 5'TTCTGCGACATGGAAGC | prJS893: 5'GGGTTTATTAAATGATTG AACAAGATGGATTGC | Amplification of JC43 backbone without insert and promoter | Generation of pLS51 |
| prLS363: 5'CTCTTCAATGCGGCAAGAATCT GACATTGTTCTCTCACATGCT TAG | prLS364: 5'ATGGGAGAACATATG GCACTTCTCGCGACATG GAAGC | pLS51 without cfa insert | Generation of pLS60 |
| prJS1187: 5'ACCTAAAAATGGATATATCGCA ACAACAAAAACAGAAGTTACC C | prJS1188: 5'ACCAATAACTGCTTAA ACGTGATTACACTAGAAA CGATAGGG | Amplification of cfa including putative natural promotor from LSB001 | Generation of pLS80 |
| prJS436: 5'GGGCTTTGCGCCCGCGCGACA ATTTCTACAGAAGACACTGCTCAAG AAGG | prJS478: 5'ATGGGATATTAAATGATTG AACAAGATGGATTGC | Amplification of KanR of JC43 | Generation or pJS208 |
| prJS430: 5'CCATTTAAGGATAGGTTAAGA TTATACC | prJS749: 5'GGGTTTATTAAATGATTG AACAAGATGGATTGC | Amplification of PaphT of JC43 | Generation of pJS208 |
| prJS428: 5’CACCAATAACTGCTTTAAAGCTTATATATGAGCTTTACCATTTCCTATCACCTGATAGG | prJS429: 5’ACCTATCACCCTTAAATGGATATATCCCGGGATATATTTAAGACCCACTTTTCACATTTAAG | Amplification of tetR-PtetR from synthetic gene fragment | Generation of pJS208 |
| Name          | Purpose                  | Sequence                                                                                     | Cloning strategy       |
|--------------|--------------------------|----------------------------------------------------------------------------------------------|------------------------|
| LS4G2 V<sub>H</sub> | Generation of pLS06      | CGGCCGCCCACCATAGAATGCAGCTGGGTCAGTCTTGCAATGTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   | Restriction digest using NotI and MfeI |
|              |                          | ATCTTCTCTCTGCTGATGGGAGTAGTTATAGGAATCTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   |                        |
|              |                          | ATCTTCTCTCTGCTGATGGGATCTTATAGGAAATCTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   |                        |
| LS4G2 V<sub>L</sub> | Generation of pLS09      | GCGGCCGCCCACCATAGAATGCAGCAGCAGCAGCAGCAGCAGCAGCTGGGTCAGTCTTGCAATGTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   | Restriction digest using NotI and HindIII |
|              |                          | ATCTTCTCTCTGCTGATGGGATCTTATAGGAAATCTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   |                        |
|              |                          | ATCTTCTCTCTGCTGATGGGATCTTATAGGAAATCTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   |                        |
| LS5G11 V<sub>H</sub> | Generation of pLS34      | GCGGCCGCCCACCATAGAATGCAGCTGGGTCAGTCTTGCAATGTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   | Restriction digest using NotI and MfeI |
|              |                          | ATCTTCTCTCTGCTGATGGGATCTTATAGGAAATCTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   |                        |
|              |                          | ATCTTCTCTCTGCTGATGGGATCTTATAGGAAATCTTCTCTGCTGATGGGATCTTATAGGAAATCATTATATGGAAGTCAGG   |                        |

Table S4. List of synthesized DNA fragments used in this study.
| LS5G11 V. | Generation of pLS35 | GCGGCCGCCACCATGGAATCACAGACCCAGGTCCTCATGGCTCTGGGTATCTGCTGCCCTGAGACATTGAGATGTCACAGTCTCATCCTCCCTACCTGTGCAGTTGAGGAGAACGTAACTATGAGCTGCAAGTCCAGTCAGAGCCTTTTATATAGCAATCAATGAAGACTACTTGGCCTGGTACCAGCAGAAACCAGGTCAGTCTCCTAAACTGCTGATTCACTGGGCATCCACTAGGGTGATCTGGGACAGATTTCACTCTGACCTCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTCGCTCACTGGGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTCGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTGTCAGCAATATTATACCTATCGGCTCACTGGGAGGCCAGGGCTGAGCTGATTGTGTAAGTATAATCGAATTCGATATCAAGCTTTACTGTCAGGAATATTATACCTACCTACCTGAGGCAAGCAGGGCTCAGCGAAGCTCATCGGCTACAGCTTTGAGGTCCCTGATCGCTTCAGGCAACGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTGTGAAGGCTGAAGACCTGGCAGTTATTACTT
### Table S5: Accession numbers of the sequences used in this study.

| Species                  | Strain       | NCBI Accession Number |
|--------------------------|--------------|-----------------------|
| Bartonella alsatica      | CIP 105477   | NZ_CP058235           |
| Bartonella. ancashensis  | 20.00        | NZ_CP010401           |
| Bartonella apis          | PEB0149      | NZ_LXYT01000001       |
| Bartonella australis     | Aust/NH1     | NC_020300             |
| Bartonella bacilliformis | KC583        | NC_008783             |
| Bartonella birtlesii     | LL-WM9       | NZ_AIMC00000000.1     |
| Bartonella bovis         | 91-4         | NZ_CM001844           |
| Bartonella claridgeiaei  | 73           | NC_014932             |
| Bartonella doshiae       | NCTC12862    | NZ_UFTF01000001       |
| Bartonella elizabethae   | NCTC12898    | NZ_LR134527           |
| Bartonella grahamii      | as4aup       | CP001562              |
| Bartonella melophagi     | K-2C         | NZ_AIMA00000000.1     |
| Bartonella henselae      | Houston-1    | NC_005956             |
| Bartonella koehlerae     | C-29         | NZ_AHPL00000000.1     |
| Bartonella quintana      | Toulouse     | NC_005955             |
|                          | NCTC12899    | LS483373              |
|                          | JK19         | NZ_AHPH00000000.1     |
|                          | JK39         | NZ_AHPB00000000.1     |
|                          | MF1-1        | NZ_AP019773           |
|                          | RM-11        | NC_018533             |
|                          | JK31         | NZ_AHPG00000000.1     |
|                          | JK56         | NZ_AHPE00000000.1     |
|                          | JK63         | NZ_AHPF00000000.1     |
|                          | BQ2-B70      | NZ_AZZV00000000.1     |
|                          | JK67         | NZ_AHPC00000000.1     |
|                          | JK68         | NZ_AHPD00000000.1     |
|                          | JK7          | NZ_AZZZ00000000.1     |
|                          | JK12         | NZ_AZZY00000000.1     |
|                          | JK73         | NZ_AZZX00000000.1     |
|                          | JK73rel      | NZ_AZZW00000000.1     |
| Bartonella rattimassiliensis | 15908   | NZ_AILY00000000.1     |
| Bartonella rochalimae    | BMGH         | NZ_AHPK00000000.1     |
| Bartonella schoenbuchensis | R1        | NZ_CP019789           |
| Bartonella sp.           | 1-1C         | NZ_CP019489           |
|                          | AR15-3       | NZ_MUYE00000000       |
|                          | JB15         | NZ_CP019787           |
| Bartonella taylorii      | IBS296       | CP083444              |
|                          | M1           | CP083693              |
| Bartonella tamiae        | Th239        | NZ_AIMB00000000       |
| Bartonella triboorum     | CIP 105476   | NC_010161             |
| Bartonella vinsonii      | NCTC12905    | NZ_LR134529           |
| Bartonella vinsonii ssp. arupensis | Pm136co | NZ_AIMH00000000.1     |
|                          | OK-94-513    | NZ_AIZLZ00000000.1    |
| Bartonella vinsonii ssp. berkhofii | Winnie | NC_020301.1           |
|                          | Tweed        | NZ_AGW00000000.1      |
| Bartonella washoensis    | Sb944nv      | NZ_AILU00000000.1     |
| "Candidatus Tokpelia hoeldobleri" | Hsal     | CP017315              |
| Brucella melitensis      | 16M          | AE008917, AE008918    |
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